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(54) Title: METHODS FOR THE TREATMENT OF METABOLIC DISORDERS, INCLUDING OBESITY AND DIABETES

(57) Abstract: The present invention relates to methods and compositions for the diagnosis and treatment of metabolic disorders, including, but not limited to, obesity, diabetes, overweight, anorexia, or cachexia. The invention further provides methods for identifying a compound capable of treating a metabolic disorder. The invention also provides methods for identifying a compound capable of modulating a metabolic activity. Yet further, the invention provides a method for modulating a metabolic activity. In addition, the invention provides a method for treating a subject having a metabolic disorder characterized by aberrant 14273 polypeptide activity or aberrant 14273 nucleic acid expression. In another aspect, the invention provides methods for modulating lipogenesis in a subject and methods for modulating lipolysis in a subject. In yet another aspect, the invention provides methods for regulating endogenous glucose levels.

# METHODS FOR THE TREATMENT OF METABOLIC DISORDERS, INCLUDING OBESITY AND DIABETES

## Related Applications

5      The present application claims priority to U.S. provisional application serial no. 60/271,655 filed on February 26, 2001, the contents of which are expressly incorporated herein by reference.

## Background of the Invention

10     Obesity represents the most prevalent of body weight disorders, affecting an estimated 30 to 50% of the middle-aged population in the western world. Other body weight disorders, such as anorexia nervosa and bulimia nervosa, which together affect approximately 0.2% of the female population of the western world, also pose serious health threats. Further, such disorders as anorexia and cachexia (wasting) are also

15     prominent features of other diseases such as cancer, cystic fibrosis, and AIDS.

Obesity, defined as a body mass index (BMI) of 30 $kg/^2m$ or more, contributes to diseases such as coronary artery disease, hypertension, stroke, diabetes, hyperlipidemia and some cancers. (See, *e.g.*, Nishina, P.M. *et al.* (1994), *Metab.* 43:554-558; Grundy, S. M. & Barnett, J.P. (1990), Dis. Mon. 36:641-731). Obesity is a complex

20     multifactorial chronic disease that develops from an interaction of genotype and the environment and involves social, behavioral, cultural, physiological, metabolic and genetic factors.

Generally, obesity results when energy intake exceeds energy expenditure, resulting in the growth and/or formation of adipose tissue via hypertrophic and

25     hyperplastic growth. Hypertrophic growth is an increase in size of adipocytes stimulated by lipid accumulation. Hyperplastic growth is defined as an increase in the number of adipocytes in adipose tissue. It is thought to occur primarily by mitosis of pre-existing adipocytes caused when adipocytes fill with lipid and reach a critical size. An increase in the number of adipocytes has far-reaching consequences for the treatment

30     and prevention of obesity.

Adipose tissue consists primarily of adipocytes. Vertebrates possess two distinct types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT stores and releases fat according to the nutritional needs of the animal. This stored fat is used by the body for (1) heat insulation (*e.g.*, subcutaneous fat), (2)

35     mechanical cushion (*e.g.*, surrounding internal organs), and (3) as a source of energy. BAT burns fat, releasing the energy as heat through thermogenesis. BAT thermogenesis is used both (1) to maintain homeothermy by increasing thermogenesis in response to

lower temperatures and (2) to maintain energy balance by increasing energy expenditure in response to increases in caloric intake (Sears, I. B. *et al.* (1996) *Mol. Cell. Biol.* 16(7):3410-3419). BAT is also the major site of thermogenesis in rodents and plays an important role in thermogenesis in human infants. In humans, and to a lesser extend

5    rodents, brown fat diminishes with age, but can be re-activated under certain conditions, such as prolonged exposure to cold, maintenance on a high fat diet and in the presence of noradrenaline producing tumors.

Fat metabolism is regulated by two pathways, lipogenesis and lipolysis. Lipogenesis is the deposition of fat which occurs in the liver and in adipose tissue at

10   cytoplasmic and mitochondrial sites. This process allows the storage of energy that is ingested which is not needed for current energy demands. Lipolysis is the chemical decomposition and release of fat from adipose and/or other tissues. This process predominates over lipogenesis when additional energy is required by the body.

Diabetes mellitus is the most common metabolic disease worldwide. Every day,

15   1700 new cases of diabetes are diagnosed in the United States, and at least one-third of the 16 million Americans with diabetes are unaware of it. Diabetes is the leading cause of blindness, renal failure, and lower limb amputations in adults and is a major risk factor for cardiovascular disease and stroke.

Normal glucose homeostasis requires the finely tuned orchestration of insulin

20   secretion by pancreatic beta cells in response to subtle changes in blood glucose levels, delicately balanced with secretion of counter-regulatory hormones such as glucagon. One of the fundamental actions of insulin is to stimulate uptake of glucose from the blood into tissues, especially muscle and fat. Type 1 diabetes results from autoimmune destruction of pancreatic beta cells causing insulin deficiency. Type 2 or non-

25   insulin-dependent diabetes mellitus (NIDDM) accounts for >90% of cases and is characterized by a triad of (1) resistance to insulin action on glucose uptake in peripheral tissues, especially skeletal muscle and adipocytes, (2) impaired insulin action to inhibit hepatic glucose production, and (3) misregulated insulin secretion (DeFronzo, (1997) Diabetes Rev. 5:177-269). In most cases, type 2 diabetes is a polygenic disease with

30   complex inheritance patterns (reviewed in Kahn *et al.*, (1996) Annu. Rev. Med. 47:509-531).

Environmental factors, especially diet, physical activity, and age, interact with genetic predisposition to affect disease prevalence. Susceptibility to both insulin resistance and insulin secretory defects appears to be genetically determined (Kahn, *et*

35   *al., supra*). Defects in insulin action precede the overt disease and are seen in non-diabetic relatives of diabetic subjects. In spite of intense investigation, the genes responsible for the common forms of Type 2 diabetes remain unknown.

5   **Summary of the Invention**

        The present invention provides methods and compositions for the diagnosis and
treatment of metabolic disorders, *e.g.*, obesity, anorexia, cachexia, and diabetes. The
present invention is based, at least in part, on the discovery that 14273 molecules are
expressed at high levels in adipose tissue, *e.g.*, white adipose tissue (WAT) (see Figures
10  3A-3C) and brown adipose tissue (BAT) (see Figures 4 and 5), as well as in pancreatic
islets (see Figure 3B). 14273 molecules were further found to be upregulated during
exposure to cold (*i.e.*, under conditions that affect brown or white adipocyte
metabolism) (see Figure 6A), and downregulated in genetic models of obesity (see
Figure 6B). The present invention is also based, at least in part, on the discovery that
15  14273 knock-out mice, when fed a high-fat diet, gain more weight and have
significantly larger epididymal fat pads compared to wild-type mice. In addition, 14273
knock-out mice show increased levels of glucose and insulin upon fasting. 14273
deletion mice have glucose and insulin levels indistinguishable from wild-type mice
under fed conditions, suggesting that 14273 deletion mice have a defect in the regulation
20  of endogenous glucose production rather than glucose clearance. Increased endogenous
glucose production is recognized as a major abnormality in type II diabetes, and agents
which prevent this increase are sought-after for the treatment of type II diabetes.
Therefore, without intending to be limited by theory, it is believed that a 14273 agonist
might be beneficial to the treatment of obesity and/or type II diabetes by preventing fat
25  accumulation on a high fat diet and/or the increases in endogenous glucose production
which occur in type II diabetes.

        Accordingly, the present invention provides methods for the diagnosis and
treatment of metabolic disorders including but not limited to obesity, anorexia, cachexia,
and diabetes.

30      In one aspect, the invention provides methods for identifying a nucleic acid
molecule associated with a metabolic disorder, *e.g.*, obesity, anorexia, cachexia, and
diabetes. The method includes contacting a sample expressing a 14273 nucleic acid or
polypeptide molecule with a test compound and assaying the ability of the test
compound to modulate the expression of a 14273 nucleic acid molecule or the activity
35  of a 14273 polypeptide.

In another aspect, the invention provides methods for identifying a compound capable of treating a metabolic disorder, *e.g.*, obesity, anorexia, cachexia, and diabetes. The method includes assaying the ability of the compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity. In one embodiment, the ability of the

5     compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity is determined by detecting modulation of lipogenesis. In another embodiment, the ability of the compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity is determined by detecting modulation of lipolysis. In still another embodiment, the ability of the compound to modulate 14273 nucleic acid expression or 14273

10    polypeptide activity is determined by detecting modulation of hyperplastic growth. In yet another embodiment, the ability of the compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity is determined by detecting modulation of hypertrophic growth. In still another embodiment, the ability of the compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity is determined by

15    detecting regulation of endogenous glucose production. In yet another embodiment, the ability of the compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity is determined by detecting regulation of endogenous glucose levels.

      In another aspect, the invention provides methods for identifying a compound capable of modulating an adipocyte activity, *e.g.*, hyperplastic growth, hypertrophic

20    growth, or lipogenesis. The method includes contacting an adipocyte expressing a 14273 nucleic acid or polypeptide with a test compound and assaying the ability of the test compound to modulate the expression of a 14273 nucleic acid or the activity of a 14273 polypeptide.

      In still another aspect, the invention provides methods for identifying a

25    compound capable of modulating endogenous glucose levels, *e.g.*, modulating endogenous glucose production. The method includes contacting a cell expressing a 14273 nucleic acid or polypeptide with a test compound and assaying the ability of the test compound to modulate the expression of a 14273 nucleic acid or the activity of a 14273 polypeptide.

30    In another aspect, the invention provides methods for modulating an adipocyte activity, *e.g.*, hyperplastic growth, hypertrophic growth, or lipogenesis. The method includes contacting an adipocyte with a 14273 modulator, for example, an anti-14273 antibody, a 14273 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5 or a fragment thereof, a 14273 polypeptide comprising an amino acid sequence which

35    is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, a small molecule, an antisense 14273 nucleic acid

molecule, a nucleic acid molecule of SEQ ID NO:1 or 4 or a fragment thereof, or a ribozyme, in an amount effective to modulate an adipocyte activity.

In yet another aspect, the invention provides methods for modulating endogenous glucose levels, *e.g.*, modulating endogenous glucose production. The method includes contacting a cell with a 14273 modulator, for example, an anti-14273 antibody, a 14273 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5 or a fragment thereof, a 14273 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, a small molecule, an antisense 14273 nucleic acid molecule, a nucleic acid molecule of SEQ ID NO:1 or 4 or a fragment thereof, or a ribozyme, in an amount effective to modulate endogenous glucose levels.

In yet another aspect, the invention features a method for identifying a subject having a metabolic disorder characterized by aberrant 14273 polypeptide activity or aberrant 14273 nucleic acid expression, *e.g.*, obesity, anorexia, or cachexia. The method includes contacting a sample obtained from the subject with a test compound and assaying the ability of the test compound to modulate the expression of a 14273 nucleic acid or the activity of a 14273 polypeptide.

In yet another aspect, the invention features a method for treating a subject having a metabolic disorder, *e.g.*, obesity, diabetes, anorexia, or cachexia. The method includes administering to the subject a 14273 modulator, *e.g.*, in a pharmaceutically acceptable formulation or by using a gene therapy vector, in an amount effective to treat a subject having a metabolic disorder. Embodiments of this aspect of the invention include the 14273 modulator being a small molecule, an anti-14273 antibody, a 14273 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5 or a fragment thereof, a 14273 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, an antisense 14273 nucleic acid molecule, a nucleic acid molecule of SEQ ID NO:1 or 4 or a fragment thereof, or a ribozyme.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

## Brief Description of the Drawings

*Figures 1A-1B* depict the cDNA sequence and predicted amino acid sequence of human 14273. The nucleotide sequence corresponds to nucleic acids 1 to 1743 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 361 of SEQ ID

5  NO: 2. The coding region without the 5' and 3' untranslated regions of the human 14273 gene is shown in SEQ ID NO: 3.

*Figure 2* depicts the cDNA sequence and predicted amino acid sequence of murine 14273. The nucleotide sequence corresponds to nucleic acids 1 to 1560 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1 to 361 of SEQ ID

10  NO: 5. The coding region without the 5' and 3' untranslated regions of the human 14273 gene is shown in SEQ ID NO: 6.

*Figures 3A-3C* are graphs depicting Taqman data of human 14273 cDNA (SEQ ID NO:1) expression in various human tissues.

*Figure 4* is a graph depicting Taqman data of murine 14273 cDNA (SEQ ID

15  NO:4) expression in various mouse tissues.

*Figure 5* depicts a Northern blot analysis of murine 14273 (SEQ ID NO:4) expression in normal mouse tissues.

*Figure 6A* is a graph depicting the regulation of 14273 expression by cold exposure.

20  Figure 6B is a graph depicting the regulation of 14273 expression in various genetic animal models of obesity.

Figure 7 is a graph depicting increasingly larger body weights for 14273 deletion mice as compared to the wild-type control mice during a high-fat diet.

25  ## Detailed Description of the Invention

The present invention provides methods and compositions for the diagnosis and treatment of metabolic disorders, *e.g.*, obesity, diabetes, anorexia, and cachexia. The present invention is based, at least in part, on the discovery that the 14273 nucleic acid and polypeptide molecules (described in PCT application WO 00/00611, the contents of

30  which are incorporated herein by reference) are expressed at high levels in adipose tissue and pancreatic islets, are upregulated during exposure to cold, and are downregulated in genetic animal models of obesity. Without intending to be limited by mechanism, it is believed that the 14273 molecules can modulate the metabolism by (directly or indirectly) affecting the rate of lipogenesis and/or lipolysis.

35  The present invention is also based, at least in part, on the discovery that 14273 knock-out mice, when fed a high-fat diet, gain more weight and have significantly larger epididymal fat pads compared to wild-type mice. In addition, 14273 knock-out mice

show increased levels of glucose and insulin upon fasting. 14273 deletion mice have glucose and insulin levels indistinguishable from wild-type mice under fed conditions, suggesting that 14273 deletion mice have a defect in the regulation of endogenous glucose production rather than glucose clearance. Increased endogenous glucose
5  production is recognized as a major abnormality in type II diabetes, and agents which prevent this increase are sought-after for the treatment of type II diabetes. Therefore, without intending to be limited by theory, it is believed that a 14273 agonist might be beneficial to the treatment of obesity and/or type II diabetes by preventing fat accumulation on a high fat diet and/or the increases in endogenous glucose production
10 which occur in type II diabetes.

       As used herein, the term "metabolic disorder" includes a disorder, disease or condition which is caused or characterized by an abnormal metabolism (*i.e.*, the chemical changes in living cells by which energy is provided for vital processes and activities) in a subject. Metabolic disorders include diseases, disorders, or conditions
15 associated with aberrant thermogenesis or aberrant adipose cell (*e.g.*, brown or white adipose cell) content or function. Metabolic disorders can be characterized by a misregulation (*e.g.*, downregulation or upregulation) of 14273 activity. Metabolic disorders can detrimentally affect cellular functions such as cellular proliferation, growth, differentiation, or migration, cellular regulation of homeostasis, inter- or intra-
20 cellular communication; tissue function, such as liver function, muscle function, or adipocyte function; systemic responses in an organism, such as hormonal responses (*e.g.*, insulin response). Examples of metabolic disorders include obesity, diabetes, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Lawrence-Moon syndrome, Prader-Labhart-Willi syndrome, anorexia, and
25 cachexia. Obesity is defined as a body mass index (BMI) of 30 kg/$^2$m or more (National Institute of Health, Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998)). However, the present invention is also intended to include a disease, disorder, or condition that is characterized by a body mass index (BMI) of 25 kg/$^2$m or more, 26 kg/$^2$m or more, 27 kg/$^2$m or more, 28 kg/$^2$m or
30 more, 29 kg/$^2$m or more, 29.5 kg/$^2$m or more, or 29.9 kg/$^2$m or more, all of which are typically referred to as overweight (National Institute of Health, Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998)).

       As used interchangeably herein, "14273 activity," "biological activity of 14273"
35 or "functional activity of 14273," includes an activity exerted by a 14273 protein, polypeptide or nucleic acid molecule on a 14273 responsive cell or tissue, *e.g.*, adipocytes, or on a 14273 protein substrate, as determined *in vivo*, or *in vitro*, according

to standard techniques. 14273 activity can be a direct activity, such as an association with a 14273-target molecule. As used herein, a "substrate" or "target molecule" or "binding partner" is a molecule with which a 14273 protein binds or interacts in nature, such that 14273-mediated function, *e.g.*, modulation of metabolism, is achieved. A

5 14273 target molecule can be a non-14273 molecule or a 14273 protein or polypeptide. Examples of such target molecules include proteins in the same signaling path as the 14273 protein, *e.g.*, proteins which may function upstream (including both stimulators and inhibitors of activity) or downstream of the 14273 protein in a pathway involving regulation of metabolism. Alternatively, a 14273 activity is an indirect activity, such as

10 a cellular signaling activity mediated by interaction of the 14273 protein with a 14273 target molecule. The biological activities of 14273 are described herein. For example, the 14273 proteins can have one or more of the following activities: 1) modulation of fat homeostasis; 2) modulation of lipogenesis (*e.g.*, fat deposition necessary for heat insulation, mechanical cushion, and/or storage); 3) modulation of lipolysis (*e.g.*, fat

15 mobilization necessary as an energy source and/or for thermogenesis); 4) modulation of adipocyte growth (*e.g.*, hyperplastic and/or hypertrophic growth); 5) regulation of endogenous glucose production; and 6) regulation of endogenous glucose levels.

As used herein, "metabolic activity" includes an activity exerted by an adipose cell, or an activity that takes place in an adipose cell. For example, such activities

20 include cellular processes that contribute to the physiological role of adipose cells, such as lipogenesis and lipolysis and include, but are not limited to, cell proliferation, differentiation, growth, migration, programmed cell death, uncoupled mitochondrial respiration, and thermogenesis.

25 Various aspects of the invention are described in further detail in the following subsections:

I. Screening Assays:

30 The invention provides methods (also referred to herein as a "screening assays") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to 14273 polypeptides, have a stimulatory or inhibitory effect on, for example, 14273 expression or 14273 activity, or have a stimulatory or inhibitory effect on, for example, the expression or

35 activity of 14273 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 14273 polypeptide or polypeptide or biologically

active portion thereof. In another embodiment, the invention provides assays for
screening candidate or test compounds which bind to or modulate the activity of a
14273 polypeptide or polypeptide or biologically active portion thereof. The test
compounds of the present invention can be obtained using any of the numerous

5    approaches in combinatorial library methods known in the art, including: biological
libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic
library methods requiring deconvolution; the 'one-bead one-compound' library method;
and synthetic library methods using affinity chromatography selection. The biological
library approach is limited to peptide libraries, while the other four approaches are

10   applicable to peptide, non-peptide oligomer or small molecule libraries of compounds
(Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

         Examples of methods for the synthesis of molecular libraries can be found in the
art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et
al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med.*

15   *Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem.
Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in
Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

         Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992)
*Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor

20   (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP
'409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage
(Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406);
(Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.*
222:301-310); (Ladner *supra.*).

25       In one embodiment, an assay is a cell-based assay in which a cell which
expresses a 14273 polypeptide or biologically active portion thereof is contacted with a
test compound and the ability of the test compound to modulate 14273 activity is
determined. Determining the ability of the test compound to modulate 14273 activity
can be accomplished by monitoring, for example, glucose concentration, glucose uptake,

30   or glycerol release in a cell, or insulin secretion or glucagon secretion from a cell. The
cell, for example, can be of mammalian origin, *e.g.*, a liver cell, a skeletal muscle cell, or
a fat cell, such as an adipocyte.

         In another embodiment, an assay is a cell-based assay in which a cell which
expresses a constitutively active 14273 polypeptide or constitutively active portion

35   thereof is contacted with a test compound and the ability of the test compound to inhibit
14273 activity is determined. Examples of methods for the generation of constitutively
active G protein coupled receptors can be found in the art, for example in: Lupu-Meiri *et*

*al.* (2000) *J. Biol. Chem.* electronic publication; Nielsen *et al.* (2000) *Proc. Natl. Acad. Sci.* 97:10277-10281; Hsu *et al.* (2000) *Mol. Endocrinol.* 14:1257-1271; Han *et al.* (1998) *Biochemistry* 37:8253-8261; and Egan *et al.* (1998) *Ann. N. Y. Acad. Sci.* 861:136-139.

5      The ability of the test compound to modulate 14273 binding to a substrate or to bind to 14273 can also be determined. Determining the ability of the test compound to modulate 14273 binding to a substrate can be accomplished, for example, by coupling the 14273 substrate with a radioisotope, an enzymatic label, or a fluorescent label such that binding of the 14273 substrate to 14273 can be determined by detecting the labeled

10     14273 substrate in a complex. Alternatively, 14273 could be coupled with a radioisotope, enzymatic, or fluorescent label to monitor the ability of a test compound to modulate 14273 binding to a 14273 substrate in a complex. Determining the ability of the test compound to bind 14273 can be accomplished, for example, by coupling the compound with a radioisotope, enzymatic, or fluorescent label such that binding of the

15     compound to 14273 can be determined by detecting the labeled 14273 compound in a complex. For example, compounds (*e.g.*, 14273 substrates) can be labeled with $^{125}$I, $^{35}$S, $^{14}$C, or $^{3}$H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline

20     phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. Compounds can be fluorescently labeled with, for example, fluorescein, rhodamine, AMCA, or TRF, and the fluorescent label detected by exposure of the compound to a specific wavelength of light.

       It is also within the scope of this invention to determine the ability of a

25     compound (*e.g.*, a 14273 substrate) to interact with 14273 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with 14273 without the labeling of either the compound or the 14273. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate

30     at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 14273.

       In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a 14273 target molecule (*e.g.*, a 14273 substrate) with a test compound

35     and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 14273 target molecule. Determining the ability of the test compound to modulate the activity of a 14273 target molecule can be accomplished, for example,

by determining the ability of the 14273 polypeptide to bind to or interact with the 14273 target molecule.

Determining the ability of the 14273 polypeptide, or a biologically active fragment thereof, to bind to or interact with a 14273 target molecule can be

5    accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the 14273 polypeptide to bind to or interact with a 14273 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*,

10   intra-cellular $Ca^{2+}$, diacylglycerol, $IP_3$, and the like), detecting catalytic/enzymatic activity of the target using an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response.

15   In yet another embodiment, an assay of the present invention is a cell-free assay in which a 14273 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 14273 polypeptide or biologically active portion thereof is determined. Preferred biologically active portions of the 14273 polypeptides to be used in assays of the present invention include

20   fragments which participate in interactions with non-14273 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the 14273 polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 14273 polypeptide or biologically active portion thereof with a known compound which binds 14273 to form

25   an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 14273 polypeptide, wherein determining the ability of the test compound to interact with a 14273 polypeptide comprises determining the ability of the test compound to preferentially bind to 14273 or biologically active portion thereof as compared to the known compound.

30   In another embodiment, the assay is a cell-free assay in which a 14273 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 14273 polypeptide or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 14273 polypeptide can be

35   accomplished, for example, by determining the ability of the 14273 polypeptide to bind to a 14273 target molecule by one of the methods described above for determining direct binding. Determining the ability of the 14273 polypeptide to bind to a 14273 target

molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a 14273 polypeptide can be accomplished by determining the ability of the 14273 polypeptide to further modulate the activity of a downstream effector of a 14273 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a 14273 polypeptide or biologically active portion thereof with a known compound which binds the 14273 polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 14273 polypeptide, wherein determining the ability of the test compound to interact with the 14273 polypeptide comprises determining the ability of the 14273 polypeptide to preferentially bind to or modulate the activity of a 14273 target molecule.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either 14273 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 14273 polypeptide, or interaction of a 14273 polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/14273 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized micrometer plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 14273 polypeptide, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or micrometer plate wells are washed to remove any unbound components, the matrix

immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 14273 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the

5    screening assays of the invention. For example, either a 14273 polypeptide or a 14273 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 14273 polypeptide or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96

10   well plates (Pierce Chemical). Alternatively, antibodies reactive with 14273 polypeptide or target molecules but which do not interfere with binding of the 14273 polypeptide to its target molecule can be derivatized to the wells of the plate, and unbound target or 14273 polypeptide trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the

15   GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 14273 polypeptide or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 14273 polypeptide or target molecule.

In another embodiment, modulators of 14273 expression are identified in a

20   method wherein a cell is contacted with a candidate compound and the expression of 14273 mRNA or polypeptide in the cell is determined. The level of expression of 14273 mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of 14273 mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of 14273

25   expression based on this comparison. For example, when expression of 14273 mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 14273 mRNA or polypeptide expression. Alternatively, when expression of 14273 mRNA or polypeptide is less (statistically significantly less) in the presence of

30   the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 14273 mRNA or polypeptide expression. The level of 14273 mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting 14273 mRNA or polypeptide.

In yet another aspect of the invention, the 14273 polypeptides can be used as

35   "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993)

*Oncogene* 8:1693-1696; and Brent WO 94/10300), to identify other proteins, which bind to or interact with 14273 ("14273-binding proteins" or "14273-bp") and are involved in 14273 activity. Such 14273-binding proteins are also likely to be involved in the propagation of signals by the 14273 polypeptides or 14273 targets as, for example,

5       downstream elements of a 14273-mediated signaling pathway. Alternatively, such 14273-binding proteins are likely to be 14273 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a

10      14273 polypeptide is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a 14273-

15      dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain

20      the cloned gene which encodes the protein which interacts with the 14273 polypeptide.

The ability of a test compound to modulate lipolysis can be determined by performing an assay in which cells, *e.g.*, adipose cells, are enzymatically assayed for glycerol levels, for example, by using a kit (Sigma) designed for such a purpose (Gasic *et al.* (1999) *J. Biol. Chem.* 275:6770-6775). The ability of a test compound to modulate

25      lipogenesis can be determined by performing an assay in which cells, *e.g.*, adipose cells, are incubated in the presence of $^{14}$C-(U) glucose, and the incorporation of radioactive glucose into the cell is assayed, after cell lysis, by liquid scintillation (Wiese *et al.* (1995) *J. Biol. Chem.* 270:3442-3446). Alternatively, the ability of a test compound to modulate lipogenesis can be determined by performing an assay in which cells, *e.g.*,

30      adipose cells, are incubated in the presence of fluorescent fatty acids, and the incorporation of fluorescent fatty acids into the cell is assayed, either in live cells or after cell lysis, by fluorescence microscopy or autoradiography. The ability of a test compound to modulate lipogenesis can also be determined by performing an assay in which cells, *e.g.*, adipose cells, are stained with Oil Red O, and staining of triglycerides

35      associated with the cell is assayed by light microscopy.

The ability of a test compound to modulate insulin sensitivity of a cell can be determined by performing an assay in which cells, *e.g.*, adipose cells, are contacted with the test compound, *e.g.*, transformed to express the test compound; incubated with radioactively labeled glucose ($^{14}$C glucose); and treated with insulin. An increase or
5   decrease in glucose in the cells containing the test compound as compared to the control cells indicates that the test compound can modulate insulin sensitivity of the cells. Alternatively, the cells containing the test compound can be incubated with a radioactively labeled phosphate source (*e.g.*, [$^{32}$P]ATP) and treated with insulin. Phosphorylation of proteins in the insulin pathway, *e.g.*, the insulin receptor, can then be
10  measured. An increase or decrease in phosphorylation of a protein in the insulin pathway in cells containing the test compound as compared to the control cells indicates that the test compound can modulate insulin sensitivity of the cells.

The ability of a test compound to modulate glucose production may be determined using art known techniques. For example, cells, *e.g.*, primary hepatocytes,
15  may be cultured in 6 well plates (1.4 million cells per well) in 10% FBS-DMEM or, in the case of hormonal treatments, in serum-free DMEM. The medium may then be replaced with 1 ml of glucose production buffer consisting of glucose-free DMEM (pH 7.4), without phenol red, supplemented with 20 mM sodium lactate and 2 mM sodium pyruvate. After a 3 hour incubation, 0.5 ml of medium is collected and the glucose
20  concentration measured using a colorimetric glucose assay kit (Sigma). The readings are then normalized to the total protein content determined separately from the whole cell lysates.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-
25  based or a cell-free assay, and the ability of the agent to modulate the activity of a 14273 protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for obesity, diabetes, anorexia, or cachexia. Examples of animals that can be used include the transgenic mouse described in U.S. Patent No. 5,932,779 that contains a mutation in an endogenous melanocortin-4-receptor (MC4-R) gene; animals having mutations which
30  lead to syndromes that include obesity symptoms (described in, for example, Friedman, J. M. *et al.* (1991) *Mamm. Gen.* 1:130-144; Friedman, J. M. and Liebel, R. L. (1992) *Cell* 69:217-220; Bray, G. A. (1992) *Prog. Brain Res.* 93:333-341; and Bray, G. A. (1989) *Amer. J. Clin. Nutr.* 5:891-902); the animals described in Stubdal H. *et al.* (2000) *Mol. Cell Biol.* 20(3):878-82 (the mouse tubby phenotype characterized by maturity-
35  onset obesity); the animals described in Abadie J.M. *et al. Lipids* (2000) 35(6):613-20 (the obese Zucker rat (ZR), a genetic model of human youth-onset obesity and type 2 diabetes mellitus); the animals described in Shaughnessy S. *et al.* (2000) *Diabetes*

49(6):904-11 (mice null for the adipocyte fatty acid binding protein); the animals
described in Loskutoff D.J. *et al.* (2000) *Ann. N. Y. Acad. Sci.* 902:272-81 (the fat
mouse); or animals having mutations which lead to syndromes that include diabetes
(described in, for example, Alleva *et al.* (2001) J. *Clin. Invest.* 107:173-180; Arakawa *et*
5      *al.* (2001) *Br. J. Pharmacol.* 132:578-586; Nakamura *et al.* (2001) *Diabetes Res. Clin.*
*Pract.* 51:9-20; O'Harte *et al.* (2001) *Regul. Pept.* 96:95-104; Yamanouchi *et al.* (2000)
*Exp. Anim.* 49:259-266; Hoenig *et al.* (2000) *Am. J. Pathol.* 157:2143-2150; Reed *et al.*
(2000) *Metabolism* 49:1390-1394; and Clark *et al.* (2000) *J. Pharmacol. Toxicol.*
*Methods* 43:1-10). Other examples of animals that may be used include non-
10     recombinant, non-genetic animal models of obesity such as, for example, rabbit, mouse,
or rat models in which the animal has been exposed to either prolonged cold or long-
term over-eating, thereby, inducing hypertrophy of BAT and increasing BAT
thermogenesis (Himms-Hagen, J. (1990), *supra*).

        This invention further pertains to novel agents identified by the above-described
15     screening assays. Accordingly, it is within the scope of this invention to further use an
agent identified as described herein in an appropriate animal model. For example, an
agent identified as described herein (*e.g.*, a 14273 modulating agent, an antisense 14273
nucleic acid molecule, a 14273-specific antibody, or a 14273-binding partner) can be
used in an animal model to determine the efficacy, toxicity, or side effects of treatment
20     with such an agent. Alternatively, an agent identified as described herein can be used in
an animal model to determine the mechanism of action of such an agent. Furthermore,
this invention pertains to uses of novel agents identified by the above-described
screening assays for treatments as described herein.


25     II.     Predictive Medicine:

        The present invention also pertains to the field of predictive medicine in which
diagnostic assays, prognostic assays, and monitoring clinical trials are used for
prognostic (predictive) purposes to thereby treat an individual prophylactically.
Accordingly, one aspect of the present invention relates to diagnostic assays for
30     determining 14273 polypeptide and/or nucleic acid expression as well as 14273 activity,
in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby
determine whether an individual is afflicted with a disease or disorder, or is at risk of
developing a disorder, associated with aberrant or unwanted 14273 expression or
activity. The invention also provides for prognostic (or predictive) assays for
35     determining whether an individual is at risk of developing a disorder associated with
14273 polypeptide, nucleic acid expression or activity. For example, mutations in a
14273 gene can be assayed in a biological sample. Such assays can be used for

prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with 14273 polypeptide, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents
5  (*e.g.*, drugs, compounds) on the expression or activity of 14273 in clinical trials.

These and other agents are described in further detail in the following sections.


A.      Diagnostic Assays For Metabolic Disorders

An exemplary method for detecting the presence or absence of 14273
10  polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 14273 polypeptide or nucleic acid (*e.g.*, mRNA, or genomic DNA) that encodes 14273 polypeptide such that the presence of 14273 polypeptide or nucleic acid is detected in the biological sample. In another aspect, the present
15  invention provides a method for detecting the presence of 14273 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of 14273 activity such that the presence of 14273 activity is detected in the biological sample. A preferred agent for detecting 14273 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to 14273 mRNA or genomic DNA.
20  The nucleic acid probe can be, for example, the 14273 nucleic acid set forth in SEQ ID NO:1, 3, 4, or 6, or the DNA inserts of the plasmids deposited with ATCC as Accession Numbers PTA-1143, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 14273 mRNA or genomic DNA. Other suitable probes for use in
25  the diagnostic assays of the invention are described herein.

A preferred agent for detecting 14273 polypeptide is an antibody capable of binding to 14273 polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to
30  the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA
35  probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That

is, the detection method of the invention can be used to detect 14273 mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of 14273 mRNA include Northern hybridizations, *in situ* hybridizations, RT-PCR, and Taqman analyses. *In vitro*

5    techniques for detection of 14273 polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of 14273 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of 14273 polypeptide include introducing into a subject a labeled anti-14273 antibody. For example, the antibody can be labeled

10    with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

     The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a 14273 polypeptide; (ii) aberrant

15    expression of a gene encoding a 14273 polypeptide; (iii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a 14273 polypeptide, wherein a wild-type form of the gene encodes a polypeptide with a 14273 activity. "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes, but is not limited to, expression at

20    non-wild type levels (*e.g.*, over or under expression); a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed (*e.g.*, increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage); a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type

25    or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene (*e.g.*, a pattern of increased or decreased expression (as compared with wild type) in the

30    presence of an increase or decrease in the strength of the stimulus).

     In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

35      In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 14273 polypeptide, mRNA, or genomic DNA,

such that the presence of 14273 polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 14273 polypeptide, mRNA or genomic DNA in the control sample with the presence of 14273 polypeptide, mRNA or genomic DNA in the test sample.

5          The invention also encompasses kits for detecting the presence of 14273 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting 14273 polypeptide or mRNA in a biological sample; means for determining the amount of 14273 in the sample; and means for comparing the amount of 14273 in the sample with a standard. The compound or agent can be packaged in a

10     suitable container. The kit can further comprise instructions for using the kit to detect 14273 polypeptide or nucleic acid.


          **B.     Prognostic Assays For Metabolic Disorders**
          The diagnostic methods described herein can furthermore be utilized to identify

15     subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted 14273 expression or activity. As used herein, the term "aberrant" includes a 14273 expression or activity which deviates from the wild type 14273 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type

20     developmental pattern of expression or the subcellular pattern of expression. For example, aberrant 14273 expression or activity is intended to include the cases in which a mutation in the 14273 gene causes the 14273 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional 14273 polypeptide or a polypeptide which does not function in a wild-type fashion, *e.g.*, a

25     polypeptide which does not interact with a 14273 substrate, *e.g.*, a G protein coupled receptor subunit or ligand, or one which interacts with a non-14273 substrate, *e.g.* a non-G protein coupled receptor subunit or ligand. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response, such as cellular proliferation. For example, the term unwanted includes a 14273 expression or activity

30     which is undesirable in a subject.
          The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in 14273 polypeptide activity or nucleic acid expression, such as a fat metabolism disorder. Alternatively, the prognostic assays can

35     be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in 14273 polypeptide activity or nucleic acid expression, such as a fat metabolism disorder. Thus, the present invention provides a method for identifying a

disease or disorder associated with aberrant or unwanted 14273 expression or activity in which a test sample is obtained from a subject and 14273 polypeptide or nucleic acid (e.g., mRNA or genomic DNA) is detected, wherein the presence of 14273 polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or

5     disorder associated with aberrant or unwanted 14273 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

      Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist,

10    peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 14273 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a metabolism-associated disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated

15    with an agent for a disorder associated with aberrant or unwanted 14273 expression or activity in which a test sample is obtained and 14273 polypeptide or nucleic acid expression or activity is detected (e.g., wherein the abundance of 14273 polypeptide or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted 14273 expression or

20    activity).

      The methods of the invention can also be used to detect genetic alterations in a 14273 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 14273 polypeptide activity or nucleic acid expression, such as a metabolism-associated disorder. In preferred embodiments, the

25    methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 14273-polypeptide, or the mis-expression of the 14273 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 14273 gene; 2) an addition

30    of one or more nucleotides to a 14273 gene; 3) a substitution of one or more nucleotides of a 14273 gene, 4) a chromosomal rearrangement of a 14273 gene; 5) an alteration in the level of a messenger RNA transcript of a 14273 gene, 6) aberrant modification of a 14273 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 14273 gene, 8) a

35    non-wild type level of a 14273-polypeptide, 9) allelic loss of a 14273 gene, and 10) inappropriate post-translational modification of a 14273 polypeptide. As described herein, there are a large number of assays known in the art which can be used for

detecting alterations in a 14273 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos.

5    4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the 14273 gene (see Abravaya *et al.* (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of

10   collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 14273 gene under conditions such that hybridization and amplification of the 14273 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the

15   amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional

20   amplification system (Kwoh, D. Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such

25   molecules are present in very low numbers.

In an alternative embodiment, mutations in a 14273 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel

30   electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

35       In other embodiments, genetic mutations in 14273 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.*

(1996) *Human Mutation* 7: 244-255; Kozal, M. J. *et al.* (1996) *Nature Medicine* 2: 753-
759). For example, genetic mutations in 14273 can be identified in two dimensional
arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.*
Briefly, a first hybridization array of probes can be used to scan through long stretches

5  of DNA in a sample and control to identify base changes between the sequences by
making linear arrays of sequential overlapping probes. This step allows the
identification of point mutations. This step is followed by a second hybridization array
that allows the characterization of specific mutations by using smaller, specialized probe
arrays complementary to all variants or mutations detected. Each mutation array is

10  composed of parallel probe sets, one complementary to the wild-type gene and the other
complementary to the mutant gene.

     In yet another embodiment, any of a variety of sequencing reactions known in
the art can be used to directly sequence the 14273 gene and detect mutations by
comparing the sequence of the sample 14273 with the corresponding wild-type (control)

15  sequence. Examples of sequencing reactions include those based on techniques
developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger
((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a
variety of automated sequencing procedures can be utilized when performing the
diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass

20  spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.*
(1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem.*
*Biotechnol.* 38:147-159).

     Other methods for detecting mutations in the 14273 gene include methods in
which protection from cleavage agents is used to detect mismatched bases in RNA/RNA

25  or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the
art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by
hybridizing (labeled) RNA or DNA containing the wild-type 14273 sequence with
potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded
duplexes are treated with an agent which cleaves single-stranded regions of the duplex

30  such as which will exist due to basepair mismatches between the control and sample
strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA
hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In
other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with
hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched

35  regions. After digestion of the mismatched regions, the resulting material is then
separated by size on denaturing polyacrylamide gels to determine the site of mutation.
See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.*

(1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 14273 cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a 14273 sequence, *e.g.*, a wild-type 14273 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 14273 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 14273 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not

limited to, selective oligonucleotide hybridization, selective amplification, or selective

primer extension. For example, oligonucleotide primers may be prepared in which the

known mutation is placed centrally and then hybridized to target DNA under conditions

5    which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature*

324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific

oligonucleotides are hybridized to PCR amplified target DNA or a number of different

mutations when the oligonucleotides are attached to the hybridizing membrane and

hybridized with labeled target DNA.

10   Alternatively, allele specific amplification technology which depends on selective

PCR amplification may be used in conjunction with the instant invention.

Oligonucleotides used as primers for specific amplification may carry the mutation of

interest in the center of the molecule (so that amplification depends on differential

hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3'

15   end of one primer where, under appropriate conditions, mismatch can prevent, or reduce

polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable

to introduce a novel restriction site in the region of the mutation to create cleavage-based

detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain

embodiments amplification may also be performed using Taq ligase for amplification

20   (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur

only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect

the presence of a known mutation at a specific site by looking for the presence or absence

of amplification.

The methods described herein may be performed, for example, by utilizing pre-

25   packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent

described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose

patients exhibiting symptoms or family history of a metabolic disease or illness

involving a 14273 gene.

Furthermore, any cell type or tissue in which 14273 is expressed may be utilized

30   in the prognostic assays described herein.


C.     Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a

14273 polypeptide (*e.g.*, the modulation of transport of biological molecules across

35   membranes) can be applied not only in basic drug screening, but also in clinical trials.

For example, the effectiveness of an agent determined by a screening assay as described

herein to increase 14273 gene expression, polypeptide levels, or upregulate 14273

activity, can be monitored in clinical trials of subjects exhibiting decreased 14273 gene expression, polypeptide levels, or downregulated 14273 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 14273 gene expression, polypeptide levels, or downregulate 14273 activity, can be monitored in

5    clinical trials of subjects exhibiting increased 14273 gene expression, polypeptide levels, or upregulated 14273 activity. In such clinical trials, the expression or activity of a 14273 gene, and preferably, other genes that have been implicated in, for example, a 14273-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

10       For example, and not by way of limitation, genes, including 14273, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates 14273 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on metabolism-associated disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and

15   analyzed for the levels of expression of 14273 and other genes implicated in the metabolism-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of 14273

20   or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for

25   monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 14273 polypeptide, mRNA, or genomic

30   DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 14273 polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the 14273 polypeptide, mRNA, or genomic DNA in the pre-administration sample with the 14273 polypeptide, mRNA, or

35   genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of

14273 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of 14273 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, 14273 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

### D.       Electronic Apparatus Readable Media and Arrays

Electronic apparatus readable media comprising 14273 sequence information is also provided. As used herein, "14273 sequence information" refers to any nucleotide and/or amino acid sequence information particular to the 14273 molecules of the present invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like. Moreover, information "related to" said 14273 sequence information includes detection of the presence or absence of a sequence (*e.g.*, detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (*e.g.*, detection of a level of expression, for example, a quantitative detection), detection of a reactivity to a sequence (*e.g.*, detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, "electronic apparatus readable media" refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon 14273 sequence information of the present invention.

As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the 14273 sequence information.

5 A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2,

10 Sybase, Oracle, or the like, as well as in other forms. Any number of dataprocessor structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the 14273 sequence information.

By providing 14273 sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in

15 the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for

20 performing a method for determining whether a subject has a 14273-associated disease or disorder or a pre-disposition to a 14273-associated disease or disorder, wherein the method comprises the steps of determining 14273 sequence information associated with the subject and based on the 14273 sequence information, determining whether the subject has a 14273-associated disease or disorder or a pre-disposition to a 14273-

25 associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 14273-associated disease or disorder or a pre-disposition to a disease associated with a 14273 wherein the method

30 comprises the steps of determining 14273 sequence information associated with the subject, and based on the 14273 sequence information, determining whether the subject has a 14273-associated disease or disorder or a pre-disposition to a 14273-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving

35 phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a 14273-associated disease or disorder or a pre-disposition to a 14273-associated disease or disorder associated with 14273, said method comprising the steps of receiving 14273 sequence information from the subject and/or information

5    related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 14273 and/or a 14273-associated disease or disorder, and based on one or more of the phenotypic information, the 14273 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 14273-associated disease or

10   disorder or a pre-disposition to a 14273-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a business method for determining whether a subject has a 14273-associated disease or disorder or a pre-disposition to a 14273-

15   associated disease or disorder, said method comprising the steps of receiving information related to 14273 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 14273 and/or related to a 14273-associated disease or disorder, and based on one or more of the phenotypic information, the 14273

20   information, and the acquired information, determining whether the subject has a 14273-associated disease or disorder or a pre-disposition to a 14273-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention also includes an array comprising a 14273 sequence of the present

25   invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be 14273. This allows a profile to be developed showing a battery of genes specifically expressed in one or more

30   tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression *per se* and level of expression in that

35   tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell

type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay

5      to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

10      In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a 14273-associated disease or disorder, progression of 14273-associated disease or disorder, and processes, such a cellular transformation associated with the 14273-associated disease or disorder.

15      The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (e.g., ascertaining the effect of 14273 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

20      The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including 14273) that could serve as a molecular target for diagnosis or therapeutic intervention.

25      III.      Methods of Treatment of Subjects Suffering From Metabolic Disorders:
        The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 14273 expression or activity, e.g., a metabolic disorder such as obesity or diabetes. With regards to both prophylactic and therapeutic methods of

30      treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine

35      his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 14273

molecules of the present invention or 14273 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side

5 effects.

Treatment is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy,

10 ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

15 A.      Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 14273 expression or activity, by administering to the subject a 14273 or an agent which modulates 14273 expression or at least one 14273 activity. Subjects at risk for a disease which is caused

20 or contributed to by aberrant or unwanted 14273 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 14273 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 14273

25 aberrancy, for example, a 14273 molecule, 14273 agonist or 14273 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

B.      Therapeutic Methods

30 The 14273 nucleic acid molecules, fragments of 14273 polypeptides, and anti-14273 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, polypeptide, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically

35 acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and

agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

5      A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following

10    components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of

15    tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous

20    solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy

25    syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polytheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity

30    can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include

35    isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be

brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a fragment of a 14273 polypeptide or an anti-14273 antibody) in the

5   required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions,

10  the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral

15  therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the

20  composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a

25  sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

30      Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the

35  use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the

method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as

5    determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about

10   0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age

15   of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between

20   about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

25   The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*,. including heteroorganic and organometallic

30   compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable

35   forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for

example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

5        Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is

10    furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the

15    invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet

20    of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

        Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin

25    or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs

30    or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-

35    dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly

actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).
Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

### C.    Pharmacogenomics

The 14273 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 14273 activity (e.g., 14273 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) metabolism-associated disorders (e.g., proliferative disorders) associated with aberrant or unwanted 14273 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 14273 molecule or 14273 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 14273 molecule or 14273 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) Clin. Exp. Pharmacol. Physiol. 23(10-11): 983-985 and Linder, M. W. et al. (1997) Clin. Chem. 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically

significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a

5    "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their

10   individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that

15   encodes a drugs target is known (e.g., a 14273 polypeptide of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a

20   major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug.

25   These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently

30   experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of

35   ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 14273 molecule or 14273 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been

5    turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus

10    enhance therapeutic or prophylactic efficiency when treating a subject with a 14273 molecule or 14273 modulator, such as a modulator identified by one of the exemplary screening assays described herein.


IV.    Recombinant Expression Vectors and Host Cells Used in the Methods of the

15        Invention

The methods of the invention (e.g., the screening assays described herein) include the use of vectors, preferably expression vectors, containing a nucleic acid encoding a 14273 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has

20    been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and

25    episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of

30    utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent

35    functions.

The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for

5    expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the

10   host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide

15   sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion

20   proteins or peptides, encoded by nucleic acids as described herein (e.g., 14273 proteins, mutant forms of 14273 proteins, fusion proteins, and the like).

The recombinant expression vectors to be used in the methods of the invention can be designed for expression of 14273 proteins in prokaryotic or eukaryotic cells. For example, 14273 proteins can be expressed in bacterial cells such as *E. coli*, insect cells

25   (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with

30   vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification

35   of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein

from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA)

5    and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in 14273 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for 14273 proteins. In a preferred embodiment, a 14273 fusion protein

10   expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six weeks).

In another embodiment, a nucleic acid of the invention is expressed in

15   mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2,

20   cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is

25   capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid).

The methods of the invention may further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory

30   sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to 14273 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory

35   sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are

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produced under the control of a high efficiency regulatory region, the activity of which
can be determined by the cell type into which the vector is introduced. For a discussion
of the regulation of gene expression using antisense genes, see Weintraub, H. *et al.*,
Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*,
Vol. 1(1) 1986.

Another aspect of the invention pertains to the use of host cells into which a
14273 nucleic acid molecule of the invention is introduced, *e.g.*, a 14273 nucleic acid
molecule within a recombinant expression vector or a 14273 nucleic acid molecule
containing sequences which allow it to homologously recombine into a specific site of
the host cell's genome. The terms "host cell" and "recombinant host cell" are used
interchangeably herein. It is understood that such terms refer not only to the particular
subject cell but to the progeny or potential progeny of such a cell. Because certain
modifications may occur in succeeding generations due to either mutation or
environmental influences, such progeny may not, in fact, be identical to the parent cell,
but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 14273
protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or
mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other
suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via
conventional transformation or transfection techniques. As used herein, the terms
"transformation" and "transfection" are intended to refer to a variety of art-recognized
techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including
calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated
transfection, lipofection, or electroporation. Suitable methods for transforming or
transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A
Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

A host cell used in the methods of the invention, such as a prokaryotic or
eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a 14273 protein.
Accordingly, the invention further provides methods for producing a 14273 protein
using the host cells of the invention. In one embodiment, the method comprises
culturing the host cell of the invention (into which a recombinant expression vector
encoding a 14273 protein has been introduced) in a suitable medium such that a 14273
protein is produced. In another embodiment, the method further comprises isolating a
14273 protein from the medium or the host cell.

V.      Isolated Nucleic Acid Molecules Used in the Methods of the Invention

The coding sequence of the isolated human 14273 cDNA and the predicted
amino acid sequence of the human 14273 polypeptide are shown in SEQ ID NOs:1 and
2, respectively. The coding sequence of the isolated mouse 14273 cDNA and the
5   predicted amino acid sequence of the mouse 14273 polypeptide are shown in SEQ ID
NOs:4 and 5, respectively. The 14273 sequences are also described in the PCT
application WO 00/00611, the contents of which are incorporated herein by reference.

The methods of the invention include the use of isolated nucleic acid molecules
that encode 14273 proteins or biologically active portions thereof, as well as nucleic
10  acid fragments sufficient for use as hybridization probes to identify 14273-encoding
nucleic acid molecules (e.g., 14273 mRNA) and fragments for use as PCR primers for
the amplification or mutation of 14273 nucleic acid molecules. As used herein, the term
"nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic
DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated
15  using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-
stranded, but preferably is double-stranded DNA.

A nucleic acid molecule used in the methods of the present invention, e.g., a
nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 4, or a portion
thereof, can be isolated using standard molecular biology techniques and the sequence
20  information provided herein. Using all or portion of the nucleic acid sequence of SEQ
ID NO:1 or 4 as a hybridization probe, 14273 nucleic acid molecules can be isolated
using standard hybridization and cloning techniques (e.g., as described in Sambrook, J.,
Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold
Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor,
25  NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID
NO:1 or 4 can be isolated by the polymerase chain reaction (PCR) using synthetic
oligonucleotide primers designed based upon the sequence of SEQ ID NO:1 or 4.

A nucleic acid used in the methods of the invention can be amplified using
30  cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate
oligonucleotide primers according to standard PCR amplification techniques.
Furthermore, oligonucleotides corresponding to 14273 nucleotide sequences can be
prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, the isolated nucleic acid molecules used in the
35  methods of the invention comprise the nucleotide sequence shown in SEQ ID NO:1 or 4,
a complement of the nucleotide sequence shown in SEQ ID NO:1 or 4, or a portion of
any of these nucleotide sequences. A nucleic acid molecule which is complementary to

the nucleotide sequence shown in SEQ ID NO:1 or 4, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or 4 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 4 thereby forming a stable duplex.

5          In still another preferred embodiment, an isolated nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or 4 or a portion of any of this nucleotide sequence.

10          Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1 or 4, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a 14273 protein, e.g., a biologically active portion of a 14273 protein. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically

15   comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1 or 4 of an anti-sense sequence of SEQ ID NO:1 or 4 or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or 4. In one embodiment, a nucleic acid molecule used in

20   the methods of the present invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or 4.

As used herein, the term "hybridizes under stringent conditions" is intended to

25   describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to

30   those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions

35   includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly

stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C
(or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or
more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of
reduced stringency hybridization conditions includes hybridization in 4X SSC, at about

5    50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°
C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate
to the above-recited values, e.g., at 65-70°C or at 42-50°C are also intended to be
encompassed by the present invention. SSPE (1x SSPE is 0.15M NaCl, 10 mM
NaH$_2$PO$_4$, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1x SSC is 0.15M

10   NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are
performed for 15 minutes each after hybridization is complete. The hybridization
temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-
10°C less than the melting temperature ($T_m$) of the hybrid, where $T_m$ is determined
according to the following equations. For hybrids less than 18 base pairs in length,

15   $T_m$(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49
base pairs in length, $T_m$(°C) = 81.5 + 16.6(log$_{10}$[Na$^+$]) + 0.41(%G+C) - (600/N), where
N is the number of bases in the hybrid, and [Na$^+$] is the concentration of sodium ions in
the hybridization buffer ([Na$^+$] for 1xSSC = 0.165 M). It will also be recognized by the
skilled practitioner that additional reagents may be added to hybridization and/or wash

20   buffers to decrease non-specific hybridization of nucleic acid molecules to membranes,
for example, nitrocellulose or nylon membranes, including but not limited to blocking
agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS),
chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes,
in particular, an additional preferred, non-limiting example of stringent hybridization

25   conditions is hybridization in 0.25-0.5M NaH$_2$PO$_4$, 7% SDS at about 65°C, followed by
one or more washes at 0.02M NaH$_2$PO$_4$, 1% SDS at 65°C, see e.g., Church and Gilbert
(1984) Proc. Natl. Acad. Sci. USA 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).
        In preferred embodiments, the probe further comprises a label group attached
thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme,

30   or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for
identifying cells or tissue which misexpress a 14273 protein, such as by measuring a
level of a 14273-encoding nucleic acid in a sample of cells from a subject e.g., detecting
14273 mRNA levels or determining whether a genomic 14273 gene has been mutated or
deleted.

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The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or 4 due to degeneracy of the genetic code and thus encode the same 14273 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1 or 4. In another

5   embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2 or 5.

The methods of the invention further include the use of allelic variants of human and/or mouse 14273, e.g., functional and non-functional allelic variants. Functional

10  allelic variants are naturally occurring amino acid sequence variants of the human and/or mouse 14273 protein that maintain a 14273 activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

15  Non-functional allelic variants are naturally occurring amino acid sequence variants of the human and/or mouse 14273 protein that do not have a 14273 activity. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or 5, or a substitution, insertion or deletion in critical residues or critical regions of

20  the protein.
The methods of the present invention may further use non-human orthologues of the human and/or mouse 14273 protein. Orthologues of the human and/or mouse 14273 protein are proteins that are isolated from non-human organisms and possess the same 14273 activity.

25  The methods of the present invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1 or 4 or a portion thereof, in which a mutation has been introduced. The mutation may lead to amino acid substitutions at "non-essential" amino acid residues or at "essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the

30  wild-type sequence of 14273 (e.g., the sequence of SEQ ID NO:2 or 5) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the 14273 proteins of the present invention are not likely to be amenable to alteration.

Mutations can be introduced into SEQ ID NO:1 or 4 by standard techniques,

35  such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the

amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side

5    chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 14273 protein is preferably replaced with

10   another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 14273 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 14273 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 4 the encoded protein can be expressed recombinantly

15   and the activity of the protein can be determined using the assay described herein.

Another aspect of the invention pertains to the use of isolated nucleic acid molecules which are antisense to the nucleotide sequence of SEQ ID NO:1 or 4. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a

20   double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire 14273 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a 14273. The

25   term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 14273. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also

30   referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding 14273 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of 14273 mRNA, but more preferably is an oligonucleotide which

35   is antisense to only a portion of the coding or noncoding region of 14273 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding

the translation start site of 14273 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length.

An example of an antisense molecule which is complementary to a fragment of the 5' untranslated region of SEQ ID NO:1 and which also includes the start codon is a

5  nucleic acid molecule which includes nucleotides which are complementary to nucleotides 35 to 55 of SEQ ID NO:1. This antisense molecule has the following nucleotide sequence: 5' GCGCCGGGAATGTCCCCTGAA 3' (SEQ ID NO:13). An example of an antisense molecule which is complementary to a portion of the 3' untranslated region of SEQ ID NO:1 is a nucleic acid molecule which includes

10  nucleotides which are complementary to nucleotides 1109 to 1129 of SEQ ID NO:1. This antisense molecule has the following sequence: 5' TTGTCGATTATTTCTGGCTAA 3' (SEQ ID NO:14). An example of an antisense molecule which is complementary to a fragment of the 5' untranslated region of SEQ ID NO:4 and which also includes the start codon is a nucleic acid molecule which includes

15  nucleotides which are complementary to nucleotides 190 to 206 of SEQ ID NO:4. This antisense molecule has the following nucleotide sequence: 5' CCGGGCATGTCCCCTGAG 3' (SEQ ID NO:15). An example of an antisense molecule which is complementary to a fragment of the 3' untranslated region of SEQ ID NO:4 and which also includes nucleotides which are complementary to nucleotides 1263

20  to 1280 of SEQ ID NO:4. This antisense molecule has the following nucleotide sequence: 5' TCTGTTATTTCCAGCTAA 3' (SEQ ID NO:16).

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically

25  synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic

30  acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-

35  methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-

isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the

5    antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules used in the methods of the invention are

10   typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 14273 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to

15   DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that

20   they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense

25   nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule used in the methods of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA

30   in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) Nucleic Acids. Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215:327-330).

35   In still another embodiment, an antisense nucleic acid used in the methods of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA,

to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave 14273 mRNA transcripts to thereby inhibit translation of 14273 mRNA. A ribozyme having specificity for a 14273-encoding nucleic acid can be

5    designed based upon the nucleotide sequence of a 14273 cDNA disclosed herein (*i.e.*, SEQ ID NO:1 or 4). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 14273-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively,

10   14273 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, 14273 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 14273 (*e.g.*, the 14273

15   promoter and/or enhancers) to form triple helical structures that prevent transcription of the 14273 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6): 569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the 14273 nucleic acid molecules used in the

20   methods of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide

25   nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase

30   peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci.* 93:14670-675.

PNAs of 14273 nucleic acid molecules can be used in the therapeutic and diagnostic applications described herein. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example,

35   inducing transcription or translation arrest or inhibiting replication. PNAs of 14273 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when

used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. *et al.* (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

     In another embodiment, PNAs of 14273 can be modified, (*e.g.*, to enhance their
5  stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of 14273 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNAse H and
10  DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. *et al.* (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. *et al.* (1996) *supra*
15  and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then
20  coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

     In other embodiments, the oligonucleotide used in the methods of the invention
25  may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134). In addition,
30  oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

35

VI.    Isolated 14273 Proteins and Anti-14273 Antibodies Used in the Methods of the
       Invention

       The methods of the invention include the use of isolated 14273 proteins, and
biologically active portions thereof, as well as polypeptide fragments suitable for use as
5    immunogens to raise anti-14273 antibodies. In one embodiment, native 14273 proteins
can be isolated from cells or tissue sources by an appropriate purification scheme using
standard protein purification techniques. In another embodiment, 14273 proteins are
produced by recombinant DNA techniques. Alternative to recombinant expression, a
14273 protein or polypeptide can be synthesized chemically using standard peptide
10   synthesis techniques.

       As used herein, a "biologically active portion" of a 14273 protein includes a
fragment of a 14273 protein having a 14273 activity. Biologically active portions of a
14273 protein include peptides comprising amino acid sequences sufficiently identical
to or derived from the amino acid sequence of the 14273 protein, e.g., the amino acid
15   sequence shown in SEQ ID NO:2 or 5, which include fewer amino acids than the full
length 14273 proteins, and exhibit at least one activity of a 14273 protein. Typically,
biologically active portions comprise a domain or motif with at least one activity of the
14273 protein (e.g., the N-terminal region of the 14273 protein that is believed to be
involved in the regulation of apoptotic activity). A biologically active portion of a
20   14273 protein can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150,
175, 200, 250, 300 or more amino acids in length. Biologically active portions of a
14273 protein can be used as targets for developing agents which modulate a 14273
activity.

       In a preferred embodiment, the 14273 protein used in the methods of the
25   invention has an amino acid sequence shown in SEQ ID NO:2 or 5. In other
embodiments, the 14273 protein is substantially identical to SEQ ID NO:2 or 5, and
retains the functional activity of the protein of SEQ ID NO:2 or 5, yet differs in amino
acid sequence due to natural allelic variation or mutagenesis, as described in detail in
subsection V above. Accordingly, in another embodiment, the 14273 protein used in the
30   methods of the invention is a protein which comprises an amino acid sequence at least
about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%
or more identical to SEQ ID NO:2 or 5.

       To determine the percent identity of two amino acid sequences or of two nucleic
acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps
35   can be introduced in one or both of a first and a second amino acid or nucleic acid
sequence for optimal alignment and non-identical sequences can be disregarded for
comparison purposes). In a preferred embodiment, the length of a reference sequence

aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the 14273 amino acid sequence of SEQ ID NO:2 or 5 having 361

5  amino acid residues, at least 108, preferably at least 144, more preferably at least 180, more preferably at least 217, even more preferably at least 253, and even more preferably at least 289 or 325 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino

10 acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap,

15 which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which

20 has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at

25 http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci. 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120

30 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The methods of the invention may also use 14273 chimeric or fusion proteins. As used herein, a 14273 "chimeric protein" or "fusion protein" comprises a 14273 polypeptide operatively linked to a non-14273 polypeptide. An "14273 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a 14273

35 molecule, whereas a "non-14273 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 14273 protein, e.g., a protein which is different from the 14273 protein and which is

derived from the same or a different organism. Within a 14273 fusion protein the 14273 polypeptide can correspond to all or a portion of a 14273 protein. In a preferred embodiment, a 14273 fusion protein comprises at least one biologically active portion of a 14273 protein. In another preferred embodiment, a 14273 fusion protein comprises at ·

5    least two biologically active portions of a 14273 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the 14273 polypeptide and the non-14273 polypeptide are fused in-frame to each other. The non-14273 polypeptide can be fused to the N-terminus or C-terminus of the 14273 polypeptide.

For example, in one embodiment, the fusion protein is a GST-14273 fusion

10   protein in which the 14273 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 14273.

In another embodiment, this fusion protein is a 14273 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 14273 can be increased through use of a

15   heterologous signal sequence.

The 14273 fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 14273 fusion proteins can be used to affect the bioavailability of a 14273 substrate. Use of 14273 fusion proteins may be useful therapeutically for the treatment of disorders

20   caused by, for example, (i) aberrant modification or mutation of a gene encoding a 14273 protein; (ii) mis-regulation of the 14273 gene; and (iii) aberrant post-translational modification of a 14273 protein.

Moreover, the 14273-fusion proteins used in the methods of the invention can be used as immunogens to produce anti-14273 antibodies in a subject, to purify 14273

25   ligands and in screening assays to identify molecules which inhibit the interaction of 14273 with a 14273 substrate.

Preferably, a 14273 chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in

30   accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated

35   DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to

generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 14273-encoding nucleic acid can be cloned into such an expression

5      vector such that the fusion moiety is linked in-frame to the 14273 protein.

The present invention also pertains to the use of variants of the 14273 proteins which function as either 14273 agonists (mimetics) or as 14273 antagonists. Variants of the 14273 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a 14273 protein. An agonist of the 14273 proteins can retain substantially

10     the same, or a subset, of the biological activities of the naturally occurring form of a 14273 protein. An antagonist of a 14273 protein can inhibit one or more of the activities of the naturally occurring form of the 14273 protein by, for example, competitively modulating a 14273-mediated activity of a 14273 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one

15     embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 14273 protein.

In one embodiment, variants of a 14273 protein which function as either 14273 agonists (mimetics) or as 14273 antagonists can be identified by screening combinatorial

20     libraries of mutants, *e.g.*, truncation mutants, of a 14273 protein for 14273 protein agonist or antagonist activity. In one embodiment, a variegated library of 14273 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of 14273 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides

25     into gene sequences such that a degenerate set of potential 14273 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of 14273 sequences therein. There are a variety of methods which can be used to produce libraries of potential 14273 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene

30     sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential 14273 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984)

35     *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of a 14273 protein coding sequence can be
used to generate a variegated population of 14273 fragments for screening and
subsequent selection of variants of a 14273 protein. In one embodiment, a library of
coding sequence fragments can be generated by treating a double stranded PCR

5      fragment of a 14273 coding sequence with a nuclease under conditions wherein nicking
occurs only about once per molecule, denaturing the double stranded DNA, renaturing
the DNA to form double stranded DNA which can include sense/antisense pairs from
different nicked products, removing single stranded portions from reformed duplexes by
treatment with S1 nuclease, and ligating the resulting fragment library into an expression

10     vector. By this method, an expression library can be derived which encodes N-terminal,
C-terminal and internal fragments of various sizes of the 14273 protein.

Several techniques are known in the art for screening gene products of
combinatorial libraries made by point mutations or truncation, and for screening cDNA
libraries for gene products having a selected property. Such techniques are adaptable for

15     rapid screening of the gene libraries generated by the combinatorial mutagenesis of
14273 proteins. The most widely used techniques, which are amenable to high through-
put analysis, for screening large gene libraries typically include cloning the gene library
into replicable expression vectors, transforming appropriate cells with the resulting
library of vectors, and expressing the combinatorial genes under conditions in which

20     detection of a desired activity facilitates isolation of the vector encoding the gene whose
product was detected. Recursive ensemble mutagenesis (REM), a new technique which
enhances the frequency of functional mutants in the libraries, can be used in
combination with the screening assays to identify 14273 variants (Arkin and Yourvan
(1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein*

25     *Engineering* 6(3):327-331).

The methods of the present invention further include the use of anti-14273
antibodies. An isolated 14273 protein, or a portion or fragment thereof, can be used as
an immunogen to generate antibodies that bind 14273 using standard techniques for
polyclonal and monoclonal antibody preparation. A full-length 14273 protein can be

30     used or, alternatively, antigenic peptide fragments of 14273 can be used as immunogens.
The antigenic peptide of 14273 comprises at least 8 amino acid residues of the amino
acid sequence shown in SEQ ID NO:2 or 5 and encompasses an epitope of 14273 such
that an antibody raised against the peptide forms a specific immune complex with the
14273 protein. Preferably, the antigenic peptide comprises at least 10 amino acid

35     residues, more preferably at least 15 amino acid residues, even more preferably at least
20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of 14273 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A 14273 immunogen is typically used to prepare antibodies by immunizing a
5  suitable subject, (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed 14273 protein or a chemically synthesized 14273 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with
10  an immunogenic 14273 preparation induces a polyclonal anti-14273 antibody response.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a 14273. Examples of immunologically active portions of immunoglobulin
15  molecules include F(ab) and F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind 14273 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of
20  immunoreacting with a particular epitope of 14273. A monoclonal antibody composition thus typically displays a single binding affinity for a particular 14273 protein with which it immunoreacts.

Polyclonal anti-14273 antibodies can be prepared as described above by immunizing a suitable subject with a 14273 immunogen. The anti-14273 antibody titer
25  in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 14273. If desired, the antibody molecules directed against 14273 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization,
30  *e.g.*, when the anti-14273 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc.*
35  *Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and*

*Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology
for producing monoclonal antibody hybridomas is well known (see generally Kenneth,
R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum
Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.*
5    54:387-402; Gefter, M. L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an
immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes)
from a mammal immunized with a 14273 immunogen as described above, and the
culture supernatants of the resulting hybridoma cells are screened to identify a
hybridoma producing a monoclonal antibody that binds 14273.

10      Any of the many well known protocols used for fusing lymphocytes and
immortalized cell lines can be applied for the purpose of generating an anti-14273
monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.*
(1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the
ordinarily skilled worker will appreciate that there are many variations of such methods
15    which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line)
is derived from the same mammalian species as the lymphocytes. For example, murine
hybridomas can be made by fusing lymphocytes from a mouse immunized with an
immunogenic preparation of the present invention with an immortalized mouse cell line.
Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture
20    medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any
of a number of myeloma cell lines can be used as a fusion partner according to standard
techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines.
These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse
myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG").
25    Hybridoma cells resulting from the fusion are then selected using HAT medium, which
kills unfused and unproductively fused myeloma cells (unfused splenocytes die after
several days because they are not transformed). Hybridoma cells producing a
monoclonal antibody of the invention are detected by screening the hybridoma culture
supernatants for antibodies that bind 14273, *e.g.*, using a standard ELISA assay.

30      Alternative to preparing monoclonal antibody-secreting hybridomas, a
monoclonal anti-14273 antibody can be identified and isolated by screening a
recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display
library) with 14273 to thereby isolate immunoglobulin library members that bind 14273.
Kits for generating and screening phage display libraries are commercially available
35    (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and
the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally,
examples of methods and reagents particularly amenable for use in generating and

screening antibody display library can be found in, for example, Ladner *et al.* U.S.
Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619;
Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT
International Publication WO 92/20791; Markland *et al.* PCT International Publication

5    No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288;
McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT
International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication
No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992)
*Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281;

10   Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-
896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad.
Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377;
Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc.
Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* (1990) *Nature* 348:552-554.

15            Additionally, recombinant anti-14273 antibodies, such as chimeric and
humanized monoclonal antibodies, comprising both human and non-human portions,
which can be made using standard recombinant DNA techniques, are within the scope of
the methods of the invention. Such chimeric and humanized monoclonal antibodies can
be produced by recombinant DNA techniques known in the art, for example using

20   methods described in Robinson *et al.* International Application No. PCT/US86/02269;
Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent
Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger
*et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No.
4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988)

25   *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu
*et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA*
84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985)
*Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison,
S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S.

30   Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988)
*Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

              An anti-14273 antibody can be used to detect 14273 protein (*e.g.*, in a cellular
lysate or cell supernatant) in order to evaluate the abundance and pattern of expression
of the 14273 protein. Anti-14273 antibodies can be used diagnostically to monitor

35   protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example,
determine the efficacy of a given treatment regimen. Detection can be facilitated by
coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of

detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes
5    include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include
10    $^{125}$I, $^{131}$I, $^{35}$S or $^{3}$H.


      This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence
15    Listing are incorporated herein by reference.


                                  EXAMPLES


          EXAMPLE 1:          14273 GENE EXPRESSION IN HUMAN AND MOUSE
20                                    TISSUES


          Tissues were collected from 7 week old female C57/B16J mice (control panel) and 6 week old male C57/B16J mice housed at either 4 °C or room temperature for 12 hours prior to tissue collection, or from 10 week old ob, agouti, or wild type control
25    male mice (average body weights: 49.9, 30.3, and 25.6 g, respectively) (Jackson Labs, Bar Harbor, Maine). Human RNA (adipose tissue and adipocyte samples) was purchased from Zen-Bio, Inc. (Research Triangle Park, NC) or Clontech (Palo Alto, CA), or was prepared from other available tissue samples. Total RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA.
30    cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control 18S gene, confirming efficient removal of genomic DNA contamination. 14273 expression was measured by TaqMan quantitative PCR analysis, performed according to the manufacturer's directions (Perkin Elmer Applied
35    Biosystems, Foster City, CA).

Tissue samples included the following normal human tissues: aorta, heart, veins, spinal cord, brain (cortex), glial cells, breast, ovary, pancreas, prostate, colon, kidney, liver, lung, spleen, tonsil, lymph node, thymus, skeletal muscle, skin, adipose, osteoblasts, osteoclasts, pancreatic islets, placenta, primary adipocytes, subcutaneous
5   adipose adipocytes differentiated *in vitro*, preadipocytes, brain, and small intestine.

Normal mouse tissues examined included the following: brown adipose tissue (BAT), white adipose tissue (WAT), brain (hypothalamus), skeletal muscle, liver, kidney, heart, and spleen.

PCR probes were designed by PrimerExpress software (PE Biosystems) based
10   on the respective sequences of murine and human 14273. The following probes and primers were used:

m14273 forward primer: 5' ACTTCAAGGAAAGCCCACCA 3' (SEQ ID NO:7)

m14273 reverse primer: 5' TCCGTAGATGCCTGCTGTTG 3' (SEQ ID NO:8)

15   m14273 probe: 5' TGCGCCCTGCTTTAAAAATACCCGACT 3' (SEQ ID NO:9)

h14273 forward primer: 5' ACCTGGGAGGCAGAGGTTG 3' (SEQ ID NO:10)

h14273 reverse primer: 5' TCTTGTTGCCCTGGTTGGAG 3' (SEQ ID NO:11)

h14273 probe: 5' AGTGAGCCGAGATCGTGCCATTGC 3' (SEQ ID NO:12)

20

To standardize the results between the different tissues, two probes, distinguished by different fluorescent labels, were added to each sample. The differential labeling of the probe for the 14273 and the probe for 18S RNA (as an internal control) thus enabled their simultaneous measurement in the same well.
25   Forward and reverse primers and the probes for both 18S RNA and human or murine 14273 were added to the TaqMan Universal PCR Master Mix (PE Applied Biosystems). Although the final concentration of primer and probe could vary, each was internally consistent within a given experiment. A typical experiment contained 200 nM each of the forward and reverse primers and 100 nM of the probe for the 18S RNA, as well as
30   600 nM of each of the forward and reverse primers and 200 nM of the probe for 14273. TaqMan matrix experiments were carried out using an ABI PRISM 770 Sequence Detection System (PE Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 minutes at 50 °C and 10 minutes at 95 °C, followed by two-step PCR for 40 cycles of 95 °C for 15 seconds, followed by 60 °C for 1 minute.
35   The following method was used to quantitatively calculate 14273 gene expression in the tissue samples, relative to the 18S RNA expression in the same tissue. The threshold values at which the PCR amplification started were determined using the

manufacturer's software.  PCR cycle number at threshold value was designated as CT.
Relative expression was calculated as $2^{-((CTtest-CT18S)\text{ tissue of interest} - (CTtest-CT18S)\text{ lowest expressing tissue in panel})}$.  Samples were run in duplicate and the averages of 2 relative expression
levels that were linear to the amount of template cDNA with a slope similar to the slope
5   for the internal control 18S were used.

          14273 expression in mouse tissues was also measured by Northern blot analysis,
performed with 32P-labeled DNA probes using rapid-HYB buffer (Amersham).  The
tissues examined included BAT, WAT, pancreas, kidney, heart, brain, spleen, lung,
liver, skeletal muscle, and smooth muscle.

10        The results of expression of 14273 in human tissues by TaqMan analysis showed
high levels of expression of 14273 in brain, colon, lung, adipose tissue, and pancreatic
islets (Figures 3A-3B).  14273 mRNA was present in whole adipose tissue as well as in
primary adipocytes and *in vitro* differentiated adipocytes, but was not detected in pre-
adipocytes (Figure 3C).  Furthermore, 14273 was present in pancreatic islets at
15   considerably higher levels compared to whole pancreas (Figure 3B).  These data indicate
that 14273 is preferentially expressed in tissues relevant to metabolic disease, such as
pancreatic islets and white adipocytes.

          ·   TaqMan analysis was also performed in mouse tissues as indicated above.
14273 was highly expressed in both brown and white adipose tissue, but was present at
20   considerably lower levels in most other tissues tested (Figure 4).  To confirm the
TaqMan expression data, Northern blot analysis was performed using a probe containing
195 nucleotides of the 5' untranslated region (UTR) as well as the first 735 nucleotides
of the open reading frame (ORF) of the mouse 14273 gene.  Figure 5 shows a 1.3 kD
band corresponding to 14273 that was present in brown and white adipose tissue, but
25   was undetectable in several other tissues including pancreas, kidney, heart, brain, spleen,
liver, skeletal muscle, and smooth muscle.  A strong band of a somewhat lower
molecular weight (approximately 1 kD) was present in lung.  These data are in
agreement with the TaqMan expression data and demonstrate that both white and brown
adipose tissue are major sites of 14273 expression.

30


               **EXAMPLE 2:       REGULATION OF m14273 EXPRESSION**


          To determine whether 14273 expression is regulated under conditions that affect
35   brown or white adipocyte metabolism, expression of 14273 was measured in tissues of
mice exposed to the cold for 12 hours.  Upon exposure to cold, the mice exhibited an
increase in thermogenesis in brown adipose tissue, as evidenced by an increase in UCP1

expression, while white adipose tissue showed an increase in lipolysis. As determined by TaqMan analysis (using the protocols described above in Example 1), 14273 mRNA was increased 3-fold in the BAT of mice exposed to cold, and was marginally increased in the WAT (Figure 6A). Expression of 14273 was also tested in white adipose tissue of genetically obese mice. Figure 6B shows that expression of 14273 was decreased 3-fold in the WAT of leptin-deficient ob/ob mice. No change was observed in the WAT of agouti mice. The absence of changes in the WAT of agouti mice may be explained by the considerably lower level of adiposity in these mice.

## EXAMPLE 3:        GENERATION AND ANALYSIS OF 14273 KNOCK-OUT MICE

*Materials and Methods*

The first coding exon of the mouse 14273 gene was deleted and the 14273 gene was inserted into a vector to generate the 14273 targeting vector. The 14273 targeting vector was electroporated into ES cells and antibiotic resistant colonies were isolated and screened by PCR using forward and reverse primers based on the 14273 sequence. Positive ES cell colonies were identified by a PCR fragment specific for the targeting event. Positive cells were identified and the correct targeting event was shown by enzymatic digestion and Southern Blot analysis using a probe directed at a region of the 14273 gene that is not included in the targeting vector.

The ES cells were then injected into mouse embryos to generate knock-out mice. For Northern blot analysis, polyA RNA from the mice was separated on 1.0% Agarose Formaldehyde gels, transferred onto Nitrocellulose membrane and hybridized with a *14273* probe and a human β-actin probe (as a control). The results of the Northern blot analysis demonstrate the absence of 14273 mRNA in the homozygous knock-out mice.

For Western blot analysis equal amounts of protein from the mice were separated on a 10% SDS-polyacrylamide gel and transferred onto a Immobilon-P (Millipore) membrane. The membrane was blocked in TBS, 0.1% Tween-20, 5% nonfat dry milk, 10% goat serum (Sigma) and incubated with a diluted primary anti-14273 antibody. Bound protein was then detected.

### Measurement of blood glucose and insulin

Blood was collected from the tail vein of overnight fasted or *ad lib* fed mice. Glucose measurements were performed using a Glucometer Elite XL (Crystal Chem Inc) according to the manufacturer's instructions. Insulin was measured on serum samples using the rat Insulin Elisa Kit (Crystal Chem Inc. catalog # INSKR020)

according to the manufacturer's instructions. Samples were evaluated against a standard curve generated with mouse insulin.

*Results*

5        Body Weight Gain on a high-fat diet for male 14273 deletion mice. 13 week old 14273 male knock-out (n=8) or age- and sex-matched wild-type control mice (n=10) were fed a high-fat diet (58% fat, Research Diets D12330) for 20 weeks and body weights were recorded weekly. After 20 weeks on a high-fat diet, mice were fasted overnight, euthanized and the fat pads were dissected and weighed. As shown in Figure
10      7, 14273 deletion mice showed increasingly larger body weights than wild-type control mice during the high-fat diet. Upon dissection, 14273 mice had significantly larger epididymal fat pads (p=0.03) compared to wild-type control mice (Table I).


        Glucose and insulin levels in 14273 mice on a high-fat diet. Blood glucose and
15      insulin levels were measured after an overnight fast after 5 or 17 weeks on a high-fat diet. At both timepoints, 14273 deletion mice showed increased glucose levels (Table II) which were more pronounced at the later timepoint. The increases in blood glucose levels were paralleled by an increase in insulin levels (Table II). In contrast, under conditions of *ad lib* feeding, 14273 deletion mice had blood glucose and insulin levels
20      indistinguishable from wild-type mice under fed conditions (Table II).


Table I: Adipose tissue weights in 14273 knock-out and wild-type control mice (mean + SEM):

|                        | Knock-out    | Wild-type    | P value (Ttest) |
|------------------------|--------------|--------------|-----------------|
| Epididymal Fat (g)     | 3.288±0.429  | 2.112±0.177  | 0.031           |
| Retroperitoneal Fat (g)| 1.251±0.258  | 0.836±0.086  | 0.163           |
| Brown Fat (g)          | 0.145±0.018  | 0.109±0.012  | 0.15            |

25

Table II: Glucose and insulin levels in 14273 knock-out and wild-type control mice (mean + SEM):

| | Knock-out | Wild-type | P value (Ttest) |
|---|---|---|---|
| Fasting glucose, 5 weeks (mg/dl) | 111.13±7.9 | 88.78±7 | 0.053 |
| Fed glucose, 5 weeks (mg/dl) | 132.25±4.03 | 129.7±7.5 | 0.766 |
| Fasting glucose, 17 weeks (mg/dl) | 114.25±6 | 84.22±9.4 | 0.018 |
| Fasting insulin, 5 weeks (pg/ml) | 2759±358 | 1749±447 | 0.099 |
| Fed insulin, 17 weeks (pg/ml) | 1884±308 | 1709±305 | 0.693 |
| Fasting insulin, 17 weeks (pg/ml) | 2672±434 | 1428±403 | 0.053 |

*Summary*

A possible role for 14273 agonists in the treatment of obesity and type II diabetes is indicated by the phenotypic analysis of 14273 knock-out mice. Mice deleted for 14273 are viable and show no obvious detrimental phenotypes. However, 14273 knock-out mice, when fed a high-fat diet, gained slightly more weight compared to wild-type mice, and had significantly larger epididymal fat pads compared to wild-type mice. In addition, 14273 knock-out mice showed increased levels of glucose and insulin upon fasting. 14273 deletion mice have glucose and insulin levels indistinguishable from wild-type mice under fed conditions, suggesting that 14273 deletion mice have a defect in the regulation of endogenous glucose production rather than glucose clearance. Consistent with this hypothesis, 14273 deletion mice have a normal glucose clearance profile in a glucose tolerance test. Increased endogenous glucose production is recognized as a major abnormality in type II diabetes, and agents which prevent this increase are sought-after for the treatment of type II diabetes.

The dysregulation in body weight and glucose homeostasis upon deletion of 14273 suggests a role for this receptor in the maintenance of normal body weight and glucose production. While the phenotypes in 14273 deletion mice are mild (no overt obesity or diabetes), there is precedent in the literature that mild phenotypes upon deletion of a GPCR can translate into large beneficial effects of an agonist for the same receptor. Most notably, beta-3-adrenergic receptor deletion causes only a small increase in fat pad weights with no overt effect on body weight, while beta-3-adrenergic receptor agonists have large effects on body weight mediated through both increases in energy expenditure and decreases in food intake. In summary, the data indicate that a 14273 agonist may be beneficial to the treatment of obesity and/or type II diabetes by

preventing fat accumulation on a high fat diet and/or the increases in endogenous glucose production which occur in type II diabetes.


## 5    Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1.    A method of identifying a nucleic acid molecule associated with a metabolic disorder comprising:

5          a) contacting a sample comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or 4; and

           b) detecting the presence of a nucleic acid molecule in said sample that hybridizes to said probe, thereby identifying a nucleic acid molecule associated with a

10   metabolic disorder.

2.    The method of claim 1, wherein said hybridization probe is detectably labeled.

15   3.    The method of claim 1, wherein said sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis and southern blotting prior to contacting with said hybridization probe.

4.    The method of claim 1, wherein said sample comprising nucleic acid

20   molecules is subjected to agarose gel electrophoresis and northern blotting prior to contacting with said hybridization probe.

5.    The method of claim 1, wherein said detecting is by *in situ* hybridization.

25   6.    A method of identifying a nucleic acid molecule associated with a metabolic disorder comprising:

           a) contacting a sample comprising nucleic acid molecules with a first and a second amplification primer, said first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or 4 and said second primer comprising at least 25

30   contiguous nucleotides from the complement of SEQ ID NO:1 or 4;

           b) incubating said sample under conditions that allow nucleic acid amplification; and

           c) detecting the presence of a nucleic acid molecule in said sample that is amplified, thereby identifying a nucleic acid molecule associated with a metabolic

35   disorder.

7.      The method of claim 6, wherein said sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis after said incubation step.


8.      The method of any one of claims 1 or 6, wherein said method is used to detect mRNA in said sample.


9.      The method of any one of claims 1 or 6, wherein said method is used to detect genomic DNA in said sample.


10.      A method of identifying a polypeptide associated with a metabolic disorder comprising:

       a) contacting a sample comprising polypeptides with a 14273 binding substance; and

       b) detecting the presence of a polypeptide in said sample that binds to said 14273 binding substance, thereby identifying a polypeptide associated with a metabolic disorder.


11.      The method of claim 10, wherein said binding substance is an antibody.


12.      The method of claim 10, wherein said binding substance is detectably labeled.


13.      A method of identifying a subject having a metabolic disorder, or at risk for developing a metabolic disorder comprising:

       a) contacting a sample obtained from said subject comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or 4; and

       b) detecting the presence of a nucleic acid molecule in said sample that hybridizes to said probe, thereby identifying a subject having a metabolic disorder, or at risk for developing a metabolic disorder.


14.      The method of claim 13, wherein said hybridization probe is detectably labeled.


15.      The method of claim 13, wherein said sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis and southern blotting prior to contacting with said hybridization probe.

16.     The method of claim 13, wherein said detecting is by *in situ* hybridization.

5       17.     A method of identifying a subject having a metabolic disorder, or at risk for developing a metabolic disorder comprising:

a) contacting a sample obtained from said subject comprising nucleic acid molecules with a first and a second amplification primer, said first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or 4 and said second

10      primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1 or 4;

b) incubating said sample under conditions that allow nucleic acid amplification; and

c) detecting the presence of a nucleic acid molecule in said sample that is

15      amplified, thereby identifying a subject having a metabolic disorder, or at risk for developing a metabolic disorder.

18.     The method of claim 17, wherein said sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis after said incubation step.

20

19.     The method of any one of claims 13 or 17, wherein said method is used to detect mRNA in said sample.

20.     The method of any one of claims 13 or 17, wherein said method is used

25      to detect genomic DNA in said sample.

21.     A method of identifying a subject having a metabolic disorder, or at risk for developing a metabolic disorder comprising:

a) contacting a sample obtained from said subject comprising polypeptides with

30      a 14273 binding substance; and

b) detecting the presence of a polypeptide in said sample that binds to said 14273 binding substance, thereby identifying a subject having a metabolic disorder, or at risk for developing a metabolic disorder.

35      22.     The method of claim 21, wherein said binding substance is an antibody.

23.    The method of claim 21, wherein said binding substance is detectably labeled.

24.    A method for identifying a compound capable of treating a metabolic disorder characterized by aberrant 14273 nucleic acid expression or 14273 polypeptide activity, comprising assaying the ability of the compound to modulate 14273 nucleic acid expression or 14273 polypeptide activity, thereby identifying a compound capable of treating a metabolic disorder characterized by aberrant 14273 nucleic acid expression or 14273 polypeptide activity.

25.    The method of claim 24, wherein the metabolic disorder is a disorder associated with aberrant lipogenesis.

26.    The method of claim 24, wherein the metabolic disorder a disorder associated with aberrant lipolysis.

27.    The method of claim 24, wherein the metabolic disorder is obesity.

28.    The method of claim 24, wherein the metabolic disorder is diabetes.

29.    The method of claim 24, wherein the ability of the compound to modulate the activity of the 14273 polypeptide is determined by detecting the induction of an intracellular second messenger.

30.    A method for treating a subject having a metabolic disorder comprising administering to the subject a 14273 modulator, thereby treating said subject having a metabolic disorder.

31.    The method of claim 30, wherein the 14273 modulator is a small molecule.

32.    The method of claim 30, wherein the metabolic disorder is a disorder associated with aberrant lipogenesis.

33.    The method of claim 30, wherein the metabolic disorder is a disorder associated with aberrant lipolysis.

34.    The method of claim 30, wherein the metabolic disorder is obesity.

35.    The method of claim 30, wherein the metabolic disorder is diabetes.

5    36.    The method of claim 30, wherein said 14273 modulator is administered in a pharmaceutically acceptable formulation.

37.    The method of claim 30, wherein said 14273 modulator is administered using a gene therapy vector.

10

38.    The method of 30, wherein the 14273 modulator is capable of modulating 14273 polypeptide activity.

39.    The method of claim 38, wherein the 14273 modulator is an anti-14273

15    antibody.

40.    The method of claim 38, wherein the 14273 modulator is a 14273 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5, or a fragment thereof.

20

41.    The method of claim 38, wherein the 14273 modulator is a 14273 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5.

25    42.    The method of claim 38, wherein the 14273 modulator is an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or 4 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS

30    at 50-65°C.

43.    The method of claim 30, wherein the 14273 modulator is capable of modulating 14273 nucleic acid expression.

35    44.    The method of claim 43, wherein the 14273 modulator is an antisense 14273 nucleic acid molecule.

45.    The method of claim 43, wherein the 14273 modulator is a ribozyme.

46.    The method of claim 43, wherein the 14273 modulator comprises the nucleotide sequence of SEQ ID NO:1 or 4, or a fragment thereof.

5

47.    The method of claim 43, wherein the 14273 modulator comprises a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5.

10    48.    The method of claim 43, wherein the 14273 modulator comprises a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5, wherein the nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or 4 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC,
15    0.1% SDS at 50-65°C.

49.    A method for identifying a compound capable of modulating an adipocyte activity comprising:
        a) contacting an adipocyte with a test compound; and
20        b) assaying the ability of the test compound to modulate the expression of a 14273 nucleic acid or the activity of a 14273 polypeptide;
thereby identifying a compound capable of modulating an adipocyte activity.

50.    The method of claim 49, wherein said adipocyte activity is hyperplastic
25    growth.

51.    The method of claim 49, wherein said adipocyte activity is hypertrophic growth.

30    52.    The method of claim 49, wherein said adipocyte activity is lipogenesis.

53.    A method for modulating an adipocyte activity comprising contacting an adipocyte with a 14273 modulator, in an amount effective to modulate an adipocyte activity.

35

54.    The method of claim 53, wherein the 14273 modulator is a small molecule.

55.     The method of claim 53, wherein said adipocyte activity is hyperplastic growth.

56.     The method of claim 53, wherein said adipocyte activity is hypertrophic growth.

57.     The method of claim 53, wherein said adipocyte activity is lipogenesis.

58.     The method of claim 53, wherein the 14273 modulator is capable of modulating 14273 polypeptide activity.

59.     The method of claim 58, wherein the 14273 modulator is an anti-14273 antibody.

60.     The method of claim 58, wherein the 14273 modulator is a 14273 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5, or a fragment thereof.

61.     The method of claim 58, wherein the 14273 modulator is a 14273 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5.

62.     The method of claim 58, wherein the 14273 modulator is an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or 4 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

63.     The method of claim 53, wherein the 14273 modulator is capable of modulating 14273 nucleic acid expression.

64.     The method of claim 63, wherein the 14273 modulator is an antisense 14273 nucleic acid molecule.

65.     The method of claim 63, wherein the 14273 modulator is a ribozyme.

66.     The method of claim 63, wherein the 14273 modulator comprises the nucleotide sequence of SEQ ID NO:1 or 4, or a fragment thereof.

67.     The method of claim 63, wherein the 14273 modulator comprises a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5.

68.     The method of claim 63, wherein the 14273 modulator comprises a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5, wherein the nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or 4 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

69.     A method for modulating glucose production in a cell, comprising contacting said cell with a 14273 modulator in an amount effective to modulate glucose production in said cell.

70.     A transgenic mouse whose genome comprises a homozygous null mutation in the endogenous 14273 gene, wherein said mouse exhibits a metabolic disorder.

71.     A method of identifying a compound capable of treating a metabolic disorder, comprising:
        administering said compound to a transgenic mouse whose genome comprises a null mutation in the endogenous 14273 gene, wherein said mouse exhibits a metabolic disorder, and determining the effect of the test compound on said mouse, thereby identifying a compound capable of treating a metabolic disorder.

72.     An isolated cell, or a purified preparation of cells from a transgenic mouse whose genome comprises a homozygous null mutation in the endogenous 14273 gene, wherein the production of functional 14273 is inhibited.

Input file Flh14273new; Output File Flh14273tra

Sequence length 1743

```
                                                     M   S   P   E   C   A   R   A   A      9
TCCGGACTAGTTCTAGACCGCTGCGGGCCGCCAGGCGCCGGGA ATG TCC CCT GAA TGC GCG CGG GCA GCG     27


   G   D   A   P   L   R   S   L   E   Q   A   N   R   T   R   F   P   F   F   S      29
  GGC GAC GCG CCC TTG CGC AGC CTG GAG CAA GCC AAC CGC ACC CGC TTT CCC TTC TTC TCC    87


   D   V   K   G   D   H   R   L   V   L   A   A   V   E   T   T   V   L   V   L      49
  GAC GTC AAG GGC GAC CAC CGG CTG GTG CTG GCC GCG GTG GAG ACA ACC GTG CTC GTG CTC   147


   I   F   A   V   S   L   L   G   N   V   C   A   L   V   L   V   A   R   R   R      69
  ATC TTT GCA GTG TCG CTG CTG GGC AAC GTG TGC GCC CTG GTG CTG GTG GCG CGC CGA CGA   207


   R   R   G   A   T   A   C   L   V   L   N   L   F   C   A   D   L   L   F   I      89
  CGC CGC GGC GCG ACT GCC TGC CTG GTA CTC AAC CTC TTC TGC GCG GAC CTG CTC TTC ATC   267


   S   A   I   P   L   V   L   A   V   R   W   T   E   A   W   L   L   G   P   V     109
  AGC GCT ATC CCT CTG GTG CTG GCC GTG CGC TGG ACT GAG GCC TGG CTG CTG GGC CCC GTT   327


   A   C   H   L   L   F   Y   V   M   T   L   S   G   S   V   T   I   L   T   L     129
  GCC TGC CAC CTG CTC TTC TAC GTG ATG ACG CTG AGC GGC AGC GTC ACC ATC CTC ACG CTG   387


   A   A   V   S   L   E   R   M   V   C   I   V   H   L   Q   R   G   V   R   G     149
  GCC GCG GTC AGC CTG GAG CGC ATG GTG TGC ATC GTG CAC CTG CAG CGC GGC GTG CGG GGT   447


   P   G   R   R   A   R   A   V   L   L   A   L   I   W   G   Y   S   A   V   A     169
  CCT GGG CGG CGG GCG CGG GCA GTG CTG CTG GCG CTC ATC TGG GGC TAT TCG GCG GTC GCC   507


   A   L   P   L   C   V   F   F   R   V   V   P   Q   R   L   P   G   A   D   Q     189
  GCT CTG CCT CTC TGC GTC TTC TTT CGA GTC GTC CCG CAA CGG CTC CCC GGC GCC GAC CAG   567


   E   I   S   I   C   T   L   I   W   P   T   I   P   G   E   I   S   W   D   V     209
  GAA ATT TCG ATT TGC ACA CTG ATT TGG CCC ACC ATT CCT GGA GAG ATC TCG TGG GAT GTC   627


   S   F   V   T   L   N   F   L   V   P   G   L   V   I   V   I   S   Y   S   K     229
  TCT TTT GTT ACT TTG AAC TTC TTG GTG CCA GGA CTG GTC ATT GTG ATC AGT TAC TCC AAA   687


   I   L   Q   I   T   K   A   S   R   K   R   L   T   V   S   L   A   Y   S   E     249
  ATT TTA CAG ATC ACA AAG GCA TCA AGG AAG AGG CTC ACG GTA AGC CTG GCC TAC TCG GAG   747


   S   H   Q   I   R   V   S   Q   Q   D   F   R   L   F   R   T   L   F   L   L     269
  AGC CAC CAG ATC CGC GTG TCC CAG CAG GAC TTC CGG CTC TTC CGC ACC CTC TTC CTC CTC   807


   M   V   S   F   F   I   M   W   S   P   I   I   I   T   I   L   L   I   L   I     289
  ATG GTC TCC TTC TTC ATC ATG TGG AGC CCC ATC ATC ATC ACC ATC CTC CTC ATC CTG ATC   867


   Q   N   F   K   Q   D   L   V   I   W   P   S   L   F   F   W   V   V   A   F     309
  CAG AAC TTC AAG CAA GAC CTG GTC ATC TGG CCG TCC CTC TTC TTC TGG GTG GTG GCC TTC   927
```

**Figure 1A**

```
   T    F   A    H   S    A    L    H   P    I    L    Y    H   H    T    L    C    R   H   E   329
  ACA  TTT GCT AAT TCA GCC CTA AAC CCC ATC CTC TAC AAC ATG ACA CTG TGC AGG AAT GAG   987

   W    K   K    I   F    C    C    F   W    F    P    E    K   G    A    I    L    T   D   T   349
  TGG  AAG AAA ATT TTT TGC TGC TTC TGG TTC CCA GAA AAG GGA GCC ATT TTA ACA GAC ACA  1047

   S    V   K    R   N    D    L    S   I    I    S    G    *      362
  TCT  GTC AAA AGA AAT GAC TTG TCG ATT ATT TCT GGC TAA  1086
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TTTTTCTTTATAGCCGAGTTTCTCACACCTGGCGAGCTGTGGCATGCTTTTAAACAGAGTTCATTTCCAGTACCCTCCA

TCAGTGCACCCTGCTTTAAGAAAATGAACCTATGCAAATAGACATCCACAGCGTCGGTAAATTAAGGGGTGATCACCAA

GTTTCATAATATTTTCCCTTTATAAAAGGATTTGTTGGCCAGGTGCAGTGGTTCATGCCTGTAATCCCAGCAGTTTGGG

AGGCTGAGGTGGGTGGATCACCTGAGGTCAGGAGTTCGAGACCAACCTGACCAACATGGTGAGACCCCCGTCTCTACTA

AAAATAAAAAAAAAAAATTAGCTGGGAGTGGTGGTGGGCACCTGTAATCCTAGCTACTTGGGAGGCTGAACCAGGAGAAT

CTCTTGAACCTGGGAGGCAGAGGTTGCAGTGAGCCGAGATCGTGCCATTGCACTCCAACCAGGGCAACAAGAGTGAAAC

TCCATCTTAAAAAAAAAAAAAAAAAAAGATTTGTTATGGGTTCCTTTTAAATGTGAACTTTTTTAGTGTGTTTGTAATATG

ATCAAATTTAATAAATATTTATTTATGACTGTTCAGCAAAAAAAAAAAAAAAAAAAAAGGGCGG

# Figure 1B

Input file 14273m; Output File 14273mtra
Sequence length 1560

TTGCCAAGCTCAGCGTAAGCCTCTTCCACTGCAATCTCACAGAAGGGGTTCATGGAGTGCTTCACAGCATCAGTGACCA

CTCCAGACTTGTCCGGCTTTACCCGAATCTTCACAGCGGAGTCGATGACCCTCTTGACAGCCACGAGCGCGCGCAGCTC

```
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CGGCATCTTCCCGGACGCGTGGGCCGGGCGCCCGGC ATG TCC TCT GAG TGT GCA CAG ACG ACG GGC     30

 P  G  P  S  H  T  L  D  Q  V  N  R  T  H  F  P  F  F  S  D       30
CCT GGT CCC TCG CAC ACC CTG GAC CAA GTC AAT CGC ACC CAC TTC CCT TTC TTC TCG GAT   90

 V  K  G  D  H  R  L  V  L  S  V  V  E  T  T  V  L  G  L  I       50
GTC AAG GGC GAC CAC CGG TTG GTG TTG AGC GTC GTG GAG ACC ACC GTT CTG GGA CTC ATC  150

 F  V  V  S  L  L  G  N  V  C  A  L  V  L  V  A  R  R  R  R       70
TTT GTC GTC TCA CTG CTG GGC AAC GTG TGT GCT CTA GTG CTG GTG GCG CGC CGT CGG CGC  210

 R  G  A  S  A  S  L  V  L  N  L  F  C  A  D  L  L  F  T  S       90
CGT GGG GCG TCA GCC AGC CTG GTG CTC AAC CTC TTC TGC GCG GAT TTG CTC TTC ACC AGC  270

 A  I  P  L  V  L  V  V  R  W  T  E  A  W  L  L  G  P  V  V      110
GCC ATC CCT CTA GTG CTC GTC GTG CGC TGG ACT GAG GCC TGG CTG TTG GGG CCC GTC GTC  330

 C  H  L  L  F  Y  V  H  T  M  S  G  S  V  T  I  L  T  L  A      130
TGC CAC CTG CTC TTC TAC GTG ATG ACA ATG AGC GGC AGC GTC ACG ATC CTC ACA CTG GCC  390

 A  V  S  L  E  R  M  V  C  I  V  R  L  R  R  G  L  S  G  P      150
GCG GTC AGC CTG GAG CGC ATG GTG TGC ATC GTG CGC CTC CGG CGC GGC TTG AGC GGC CCG  450

 G  R  R  T  Q  A  A  L  L  A  F  I  W  G  Y  S  A  L  A  A      170
GGG CGG CGG ACT CAG GCG GCA CTG CTG GCT TTC ATA TGG GGT TAC TCG GCG CTC GCC GCG  510

 L  P  L  Y  I  L  F  R  V  V  P  Q  R  L  P  G  G  D  Q  E      190
CTG CCC CTC TAC ATC TTG TTC CGC GTG GTC CCG CAG CGC CTT CCC GGC GGG GAC CAG GAA  570

 I  P  I  C  T  L  D  W  P  N  R  I  G  E  I  S  W  D  V  F      210
ATT CCG ATT TGC ACA TTG GAT TGG CCC AAC CGC ATA GGA GAA ATC TCA TGG GAT GTG TTT  630

 F  E  T  L  N  F  L  V  P  G  L  V  I  V  I  S  Y  S  K  I      230
TTT GAG ACT TTG AAC TTC CTG GTG CCG GGA CTG GTC ATT GTG ATC AGT TAC TCC AAA ATT  690

 L  Q  I  T  K  A  S  R  K  R  L  T  L  S  L  A  Y  S  E  S      250
TTA CAG ATC ACG AAA GCA TCG CGG AAG AGG CTT ACG CTG AGC TTG GCA TAC TCT GAG AGC  750

 H  Q  I  R  V  S  Q  Q  D  Y  R  L  F  R  T  L  F  L  L  M      270
CAC CAG ATC CGA GTG TCC CAA CAA GAC TAC CGA CTC TTC CGC ACG CTC TTC CTG CTC ATG  810

 V  S  F  F  I  M  W  S  P  I  I  I  T  I  L  L  I  L  I  Q      290
GTT TCC TTC TTC ATC ATG TGG AGT CCC ATC ATC ATC ACC ATC CTC CTC ATC TTG ATC CAA  870

 N  F  R  Q  D  L  V  I  W  P  S  L  P  F  W  V  V  A  F  T      310
AAC TTC CGG CAG GAC CTG GTC ATC TGG CCA TCC CTT TTC TTC TGG GTG GTG GCC TTC ACG  930

 F  A  N  S  A  L  N  P  I  L  Y  N  M  S  L  F  R  N  E  W      330
TTT GCC AAC TCT GCC CTA AAC CCC ATA CTG TAC AAC ATG TCG CTG TTC AGG AAC GAA TGG  990

 R  K  I  F  C  C  F  F  F  P  E  K  G  A  I  F  T  D  T  S      350
AGG AAG ATT TTT TGC TGC TTC TTT TTT CCA GAG AAG GGA GCC ATT TTT ACA GAT ACG TCT 1050

 V  R  R  N  D  L  S  V  I  S  S  *                              362
GTC AGG CGA AAT GAC TTG TCT GTT ATT TCC AGC TAA                                  1086
```

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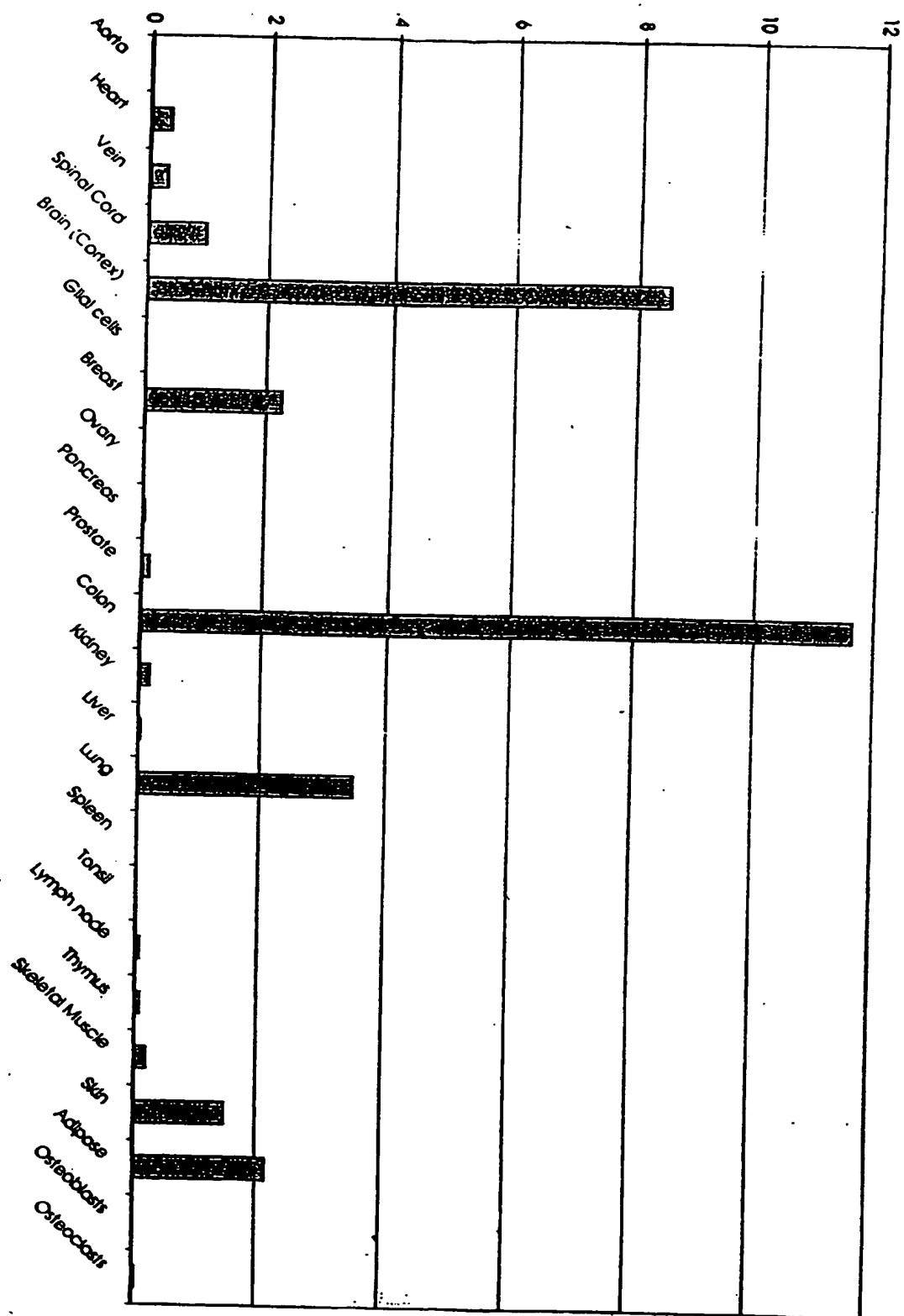
## FIGURE 2

Figure 3·A

Expression of 14273 in Human Tissues
Metabolic Phase I
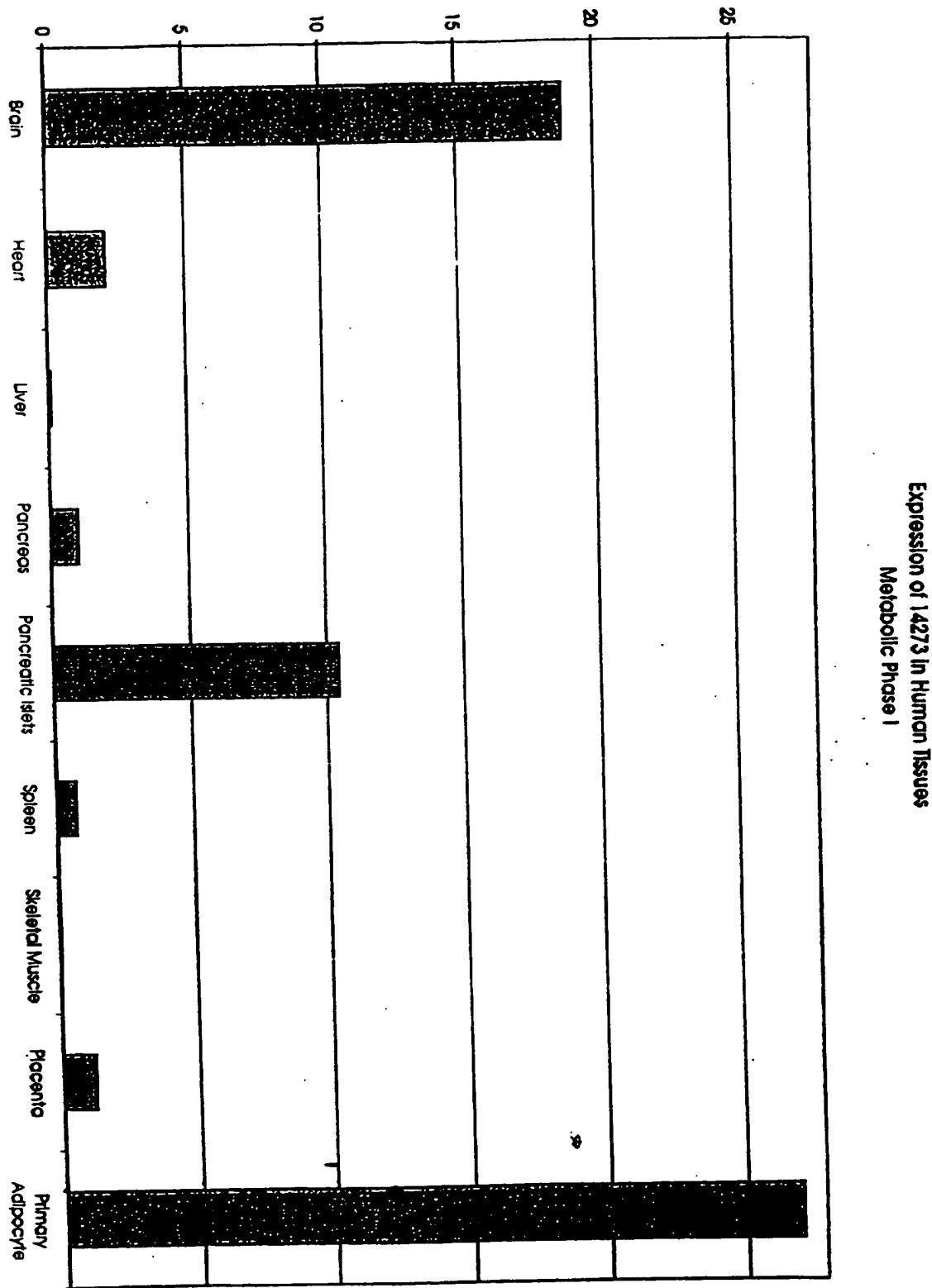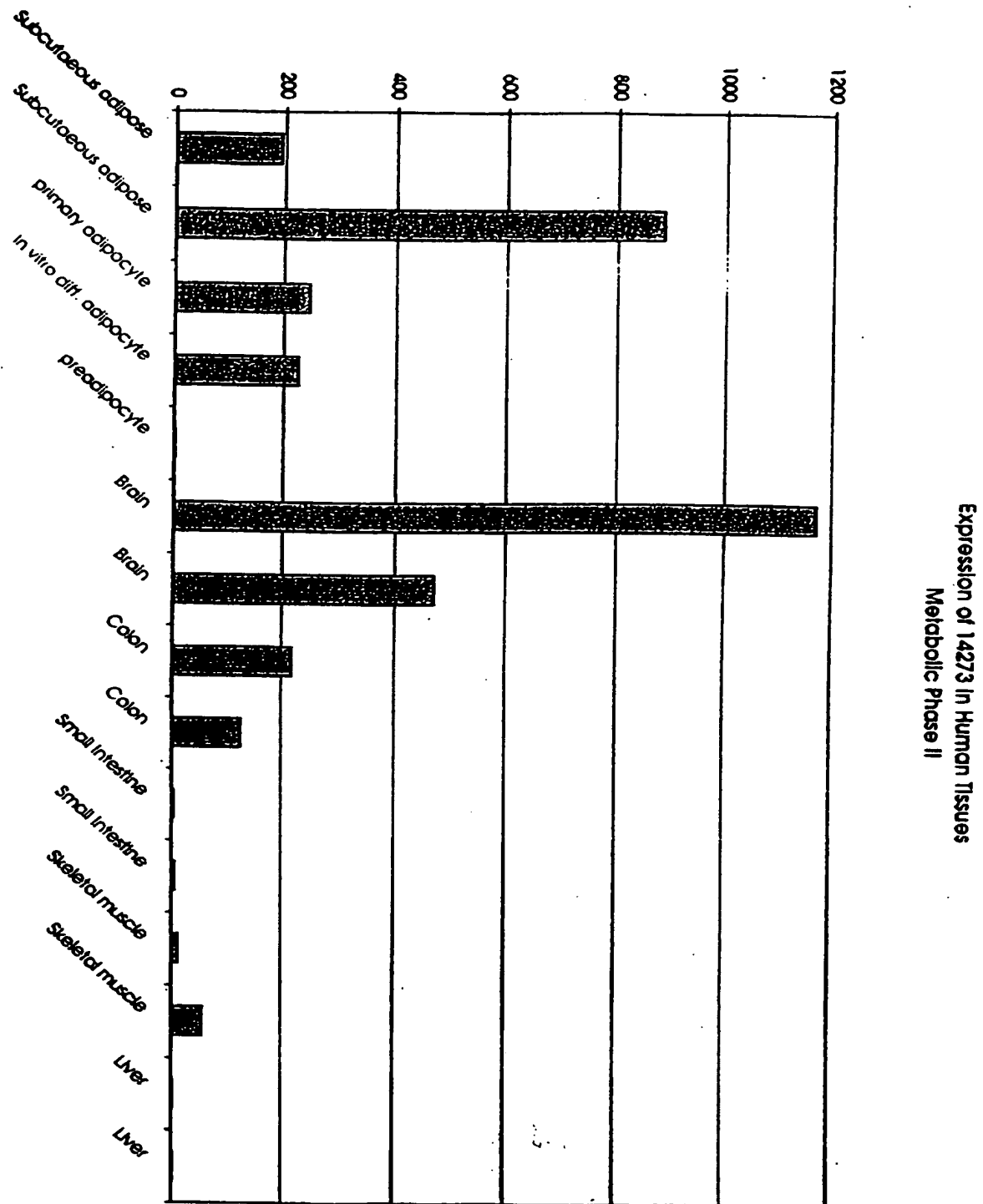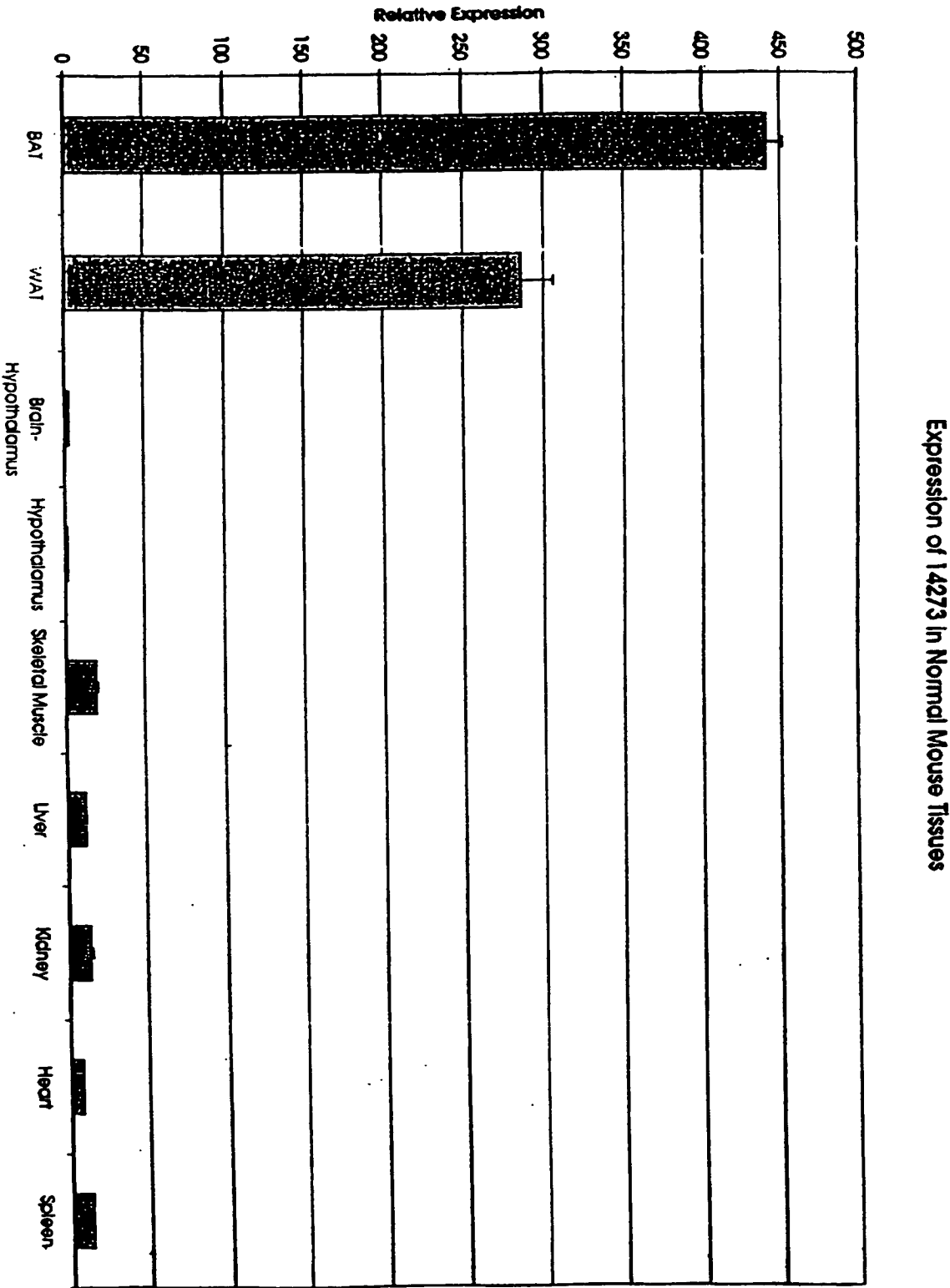
Figure 3 B

Figure 3C

Figure 4

# Expression of 14273 in Normal Mouse Tissues
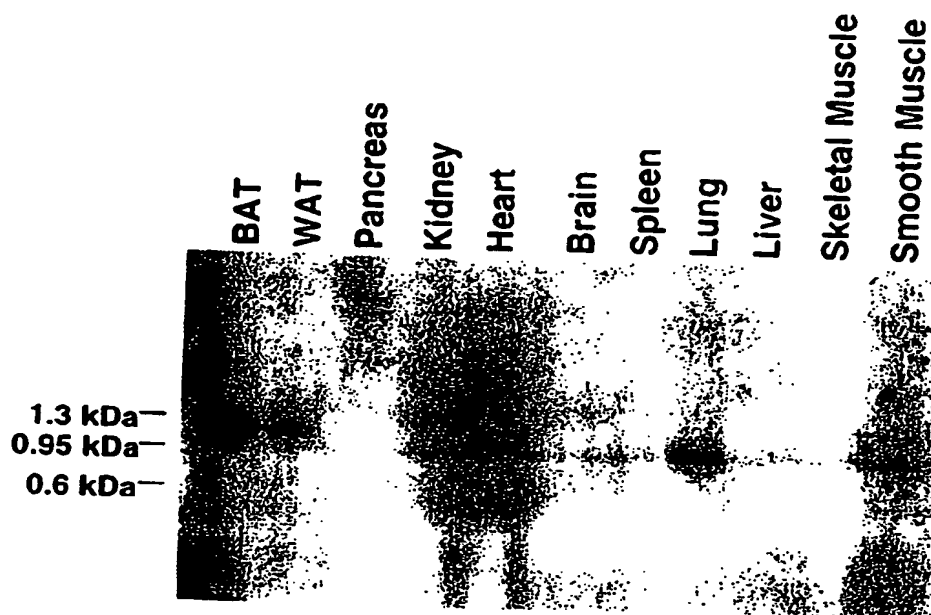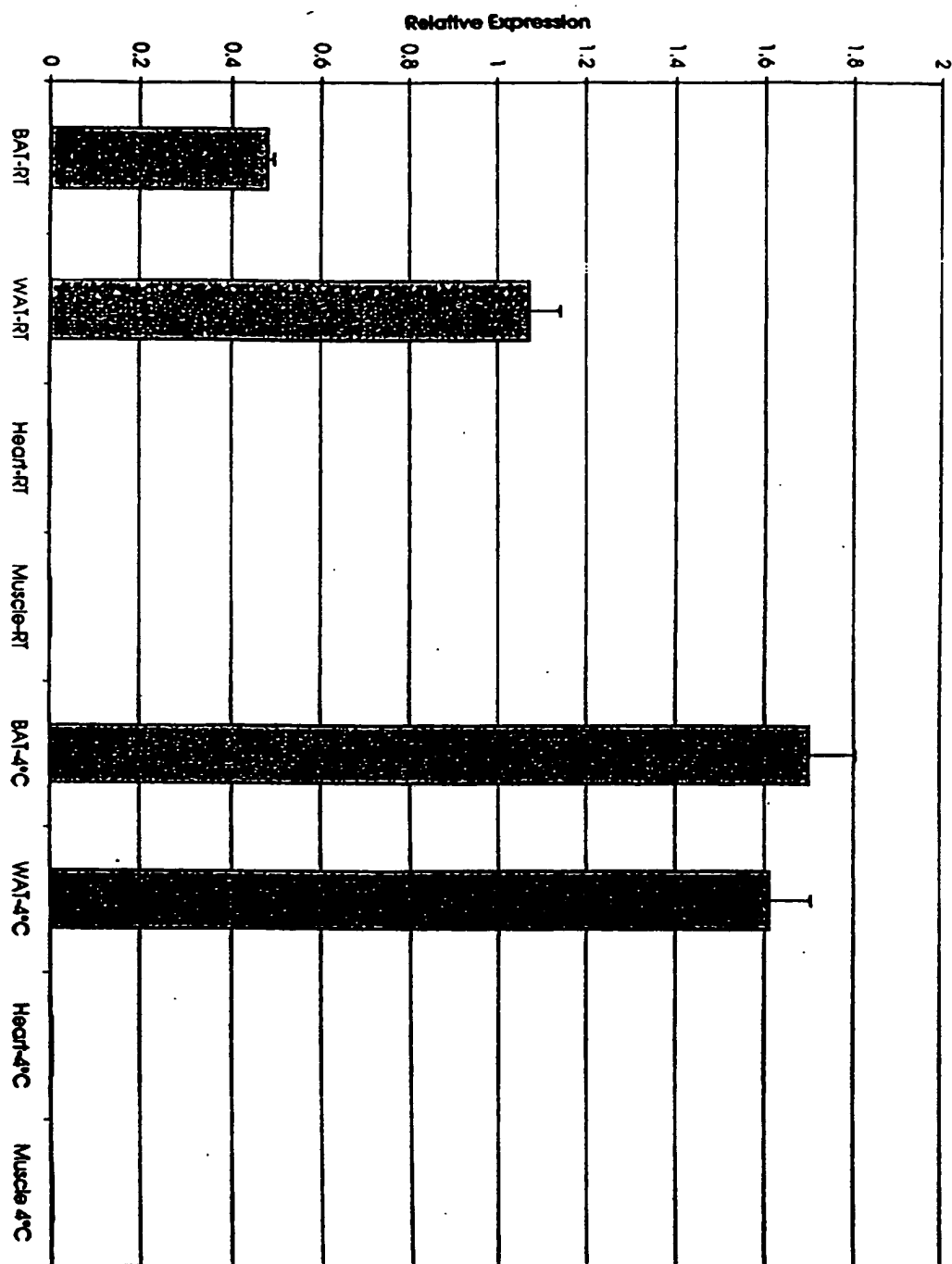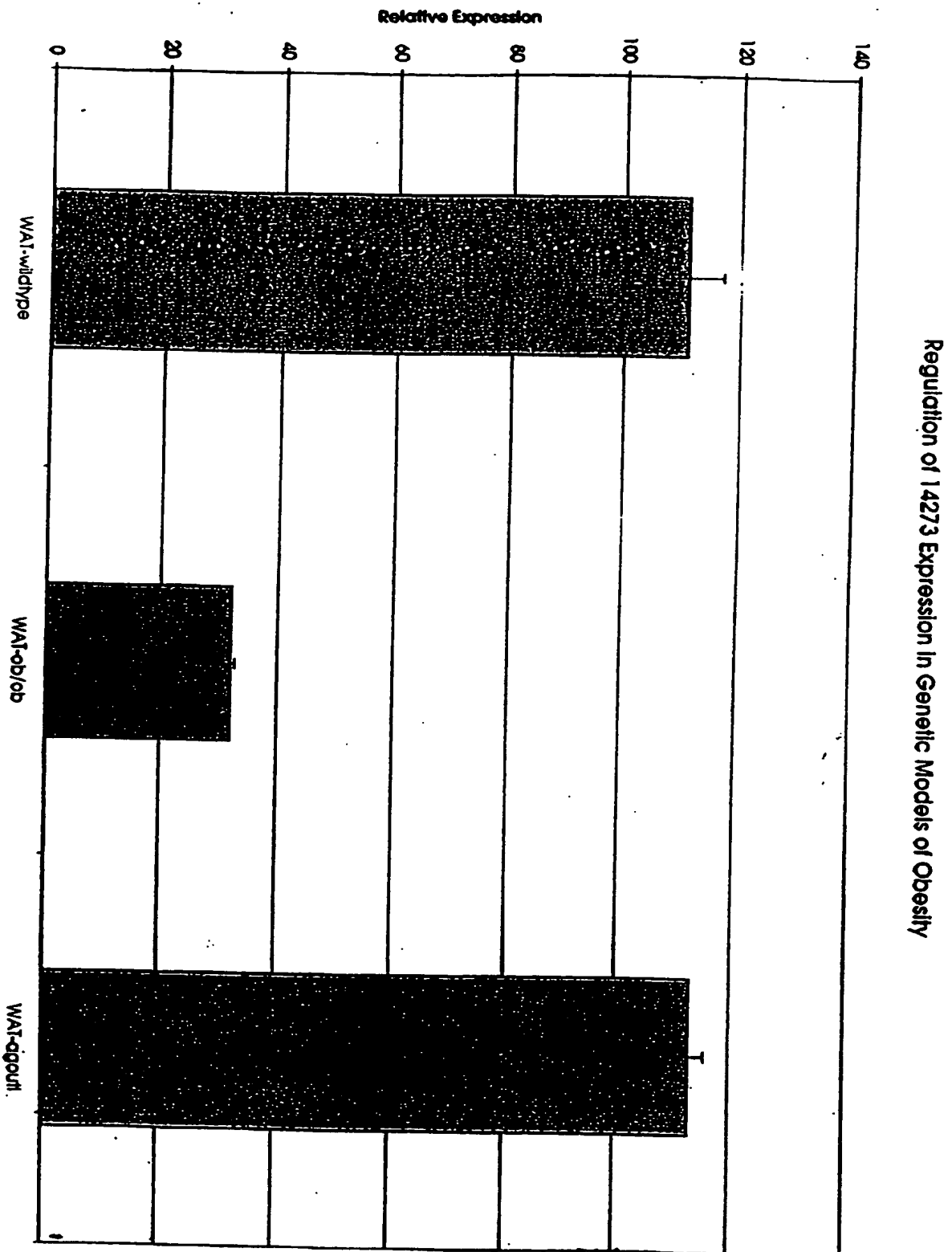


Figure 5

Figure 6A

Figure 6 B

# BW on High Fat Diet (male)

**Body weight increase of 14273 on high fat diet (male)**
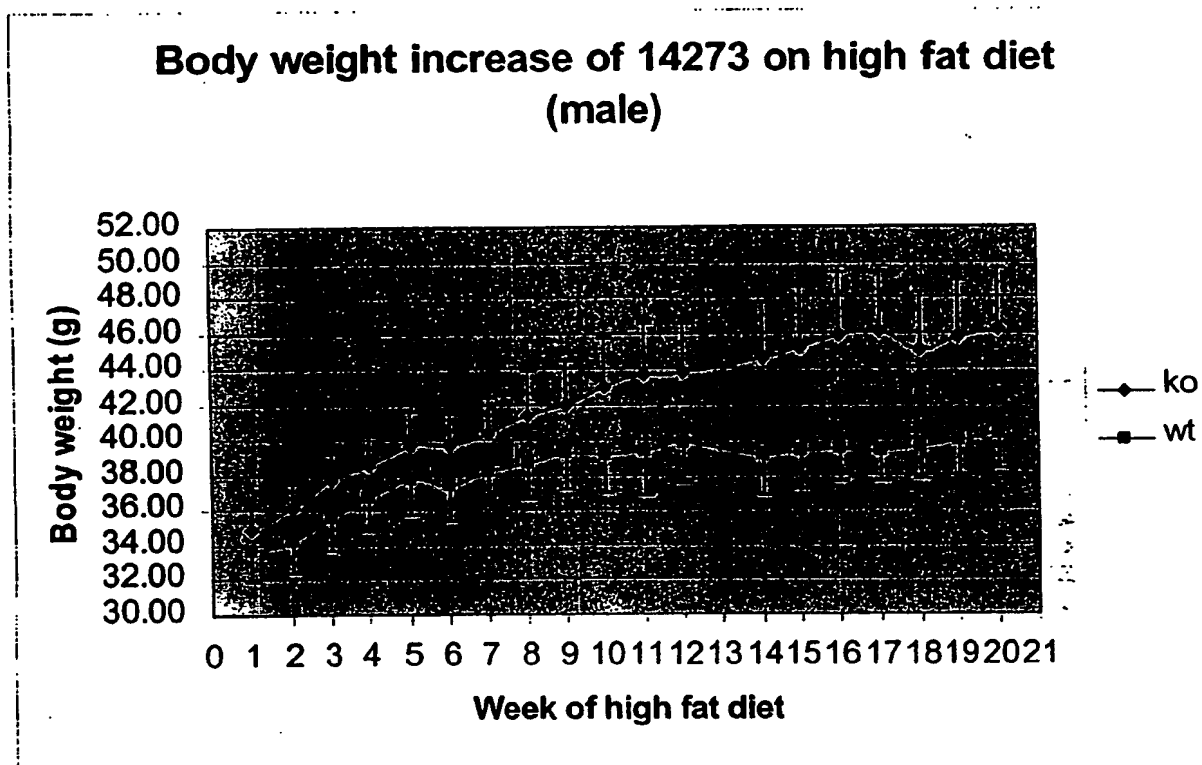


**FIGURE 7**

11/11

SEQUENCE LISTING

<110> Millennium Pharmaceuticals, Inc., et al.

<120> METHODS FOR THE TREATMENT OF METABOLIC
      DISORDERS, INCLUDING OBESITY AND DIABETES

<130> MNI-220PC

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Cys Ala Arg Ala Ala Gly Asp Ala Pro Leu Arg Ser Leu Glu Gln Ala
  5              10                  15                  20

aac cgc acc cgc ttt ccc ttc ttc tcc gac gtc aag ggc gac cac cgg    151
Asn Arg Thr Arg Phe Pro Phe Phe Ser Asp Val Lys Gly Asp His Arg
            25                  30                  35

ctg gtg ctg gcc gcg gtg gag aca acc gtg ctg gtg ctc atc ttt gca    199
Leu Val Leu Ala Ala Val Glu Thr Thr Val Leu Val Leu Ile Phe Ala
            40                  45                  50

gtg tcg ctg ctg ggc aac gtg tgc gcc ctg gtg ctg gtg gcg cgc cga    247
Val Ser Leu Leu Gly Asn Val Cys Ala Leu Val Leu Val Ala Arg Arg
            55                  60                  65

cga cgc cgc ggc gcg act gcc tgc ctg gta ctc aac ctc ttc tgc gcg    295
Arg Arg Arg Gly Ala Thr Ala Cys Leu Val Leu Asn Leu Phe Cys Ala
      70                  75                  80

gac ctg ctc ttc atc agc gct atc cct ctg gtg ctg gcc gtg cgc tgg    343
Asp Leu Leu Phe Ile Ser Ala Ile Pro Leu Val Leu Ala Val Arg Trp
85                  90                  95                  100

act gag gcc tgg ctg ctg ggc ccc gtt gcc tgc cac ctg ctc ttc tac    391
Thr Glu Ala Trp Leu Leu Gly Pro Val Ala Cys His Leu Leu Phe Tyr
                  105                 110                 115

```
gtg atg acc ctg agc ggc agc gtc acc atc ctc acg ctg gcc gcg gtc      439
Val Met Thr Leu Ser Gly Ser Val Thr Ile Leu Thr Leu Ala Ala Val
            120             125             130

agc ctg gag cgc atg gtg tgc atc gtg cac ctg cag cgc ggc gtg cgg      487
Ser Leu Glu Arg Met Val Cys Ile Val His Leu Gln Arg Gly Val Arg
            135             140             145

ggt cct ggg cgg cgg gcg cgg gca gtg ctg ctg gcg ctc atc tgg ggc      535
Gly Pro Gly Arg Arg Ala Arg Ala Val Leu Leu Ala Leu Ile Trp Gly
        150             155             160

tat tcg gcg gtc gcc gct ctg cct ctc tgc gtc ttc ttt cga gtc gtc      583
Tyr Ser Ala Val Ala Ala Leu Pro Leu Cys Val Phe Phe Arg Val Val
165             170             175             180

ccg caa cgg ctc ccc ggc gcc gac cag gaa att tcg att tgc aca ctg      631
Pro Gln Arg Leu Pro Gly Ala Asp Gln Glu Ile Ser Ile Cys Thr Leu
            185             190             195

att tgg ccc acc att cct gga gag atc tcg tgg gat gtc tct ttt gtt      679
Ile Trp Pro Thr Ile Pro Gly Glu Ile Ser Trp Asp Val Ser Phe Val
            200             205             210

act ttg aac ttc ttg gtg cca gga ctg gtc att gtg atc agt tac tcc      727
Thr Leu Asn Phe Leu Val Pro Gly Leu Val Ile Val Ile Ser Tyr Ser
            215             220             225

aaa att tta cag atc aca aag gca tca agg aag agg ctc acg gta agc      775
Lys Ile Leu Gln Ile Thr Lys Ala Ser Arg Lys Arg Leu Thr Val Ser
        230             235             240

ctg gcc tac tcg gag agc cac cag atc cgc gtg tcc cag cag gac ttc      823
Leu Ala Tyr Ser Glu Ser His Gln Ile Arg Val Ser Gln Gln Asp Phe
245             250             255             260

cgg ctc ttc cgc acc ctc ttc ctc ctc atg gtc tcc ttc ttc atc atg      871
Arg Leu Phe Arg Thr Leu Phe Leu Leu Met Val Ser Phe Phe Ile Met
            265             270             275

tgg agc ccc atc atc atc acc atc ctc ctc atc ctg atc cag aac ttc      919
Trp Ser Pro Ile Ile Ile Thr Ile Leu Leu Ile Leu Ile Gln Asn Phe
            280             285             290

aag caa gac ctg gtc atc tgg ccg tcc ctc ttc ttc tgg gtg gtg gcc      967
Lys Gln Asp Leu Val Ile Trp Pro Ser Leu Phe Phe Trp Val Val Ala
            295             300             305

ttc aca ttt gct aat tca gcc cta aac ccc atc ctc tac aac atg aca      1015
Phe Thr Phe Ala Asn Ser Ala Leu Asn Pro Ile Leu Tyr Asn Met Thr
            310             315             320

ctg tgc agg aat gag tgg aag aaa att ttt tgc tgc ttc tgg ttc cca      1063
Leu Cys Arg Asn Glu Trp Lys Lys Ile Phe Cys Cys Phe Trp Phe Pro
325             330             335             340
```

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gaa aag gga gcc att tta aca gac aca tct gtc aaa aga aat gac ttg  1111
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tcg att att tct ggc taa tttttcttta tagccgagtt tctcacacct        1159
Ser Ile Ile Ser Gly *
            360

ggcgagctgt ggcatgcttt taaacagagt tcatttccag taccctccat cagtgcaccc 1219
tgctttaaga aaatgaacct atgcaaatag acatccacag cgtcggtaaa ttaaggggtg 1279
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ggttcctttt aaatgtgaac ttttttagtg tgtttgtaat atgatcaaat ttaataaata 1699
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<213> Homo sapiens

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            20                  25                  30
Gly Asp His Arg Leu Val Leu Ala Ala Val Glu Thr Thr Val Leu Val
            35                  40                  45
Leu Ile Phe Ala Val Ser Leu Leu Gly Asn Val Cys Ala Leu Val Leu
            50                  55                  60
Val Ala Arg Arg Arg Arg Arg Gly Ala Thr Ala Cys Leu Val Leu Asn
65                  70                  75                  80
Leu Phe Cys Ala Asp Leu Leu Phe Ile Ser Ala Ile Pro Leu Val Leu
                85                  90                  95
Ala Val Arg Trp Thr Glu Ala Trp Leu Leu Gly Pro Val Ala Cys His
            100                 105                 110
Leu Leu Phe Tyr Val Met Thr Leu Ser Gly Ser Val Thr Ile Leu Thr
            115                 120                 125
Leu Ala Ala Val Ser Leu Glu Arg Met Val Cys Ile Val His Leu Gln
            130                 135                 140
Arg Gly Val Arg Gly Pro Gly Arg Arg Ala Arg Ala Val Leu Leu Ala
145                 150                 155                 160
Leu Ile Trp Gly Tyr Ser Ala Val Ala Ala Leu Pro Leu Cys Val Phe
                165                 170                 175
Phe Arg Val Val Pro Gln Arg Leu Pro Gly Ala Asp Gln Glu Ile Ser
            180                 185                 190
Ile Cys Thr Leu Ile Trp Pro Thr Ile Pro Gly Glu Ile Ser Trp Asp
            195                 200                 205
Val Ser Phe Val Thr Leu Asn Phe Leu Val Pro Gly Leu Val Ile Val
            210                 215                 220
Ile Ser Tyr Ser Lys Ile Leu Gln Ile Thr Lys Ala Ser Arg Lys Arg
225                 230                 235                 240
Leu Thr Val Ser Leu Ala Tyr Ser Glu Ser His Gln Ile Arg Val Ser
                245                 250                 255
```

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Gln Gln Asp Phe Arg Leu Phe Arg Thr Leu Phe Leu Leu Met Val Ser
        260                     265                 270
Phe Phe Ile Met Trp Ser Pro Ile Ile Ile Thr Ile Leu Leu Ile Leu
        275                 280                 285
Ile Gln Asn Phe Lys Gln Asp Leu Val Ile Trp Pro Ser Leu Phe Phe
        290                 295                 300
Trp Val Val Ala Phe Thr Phe Ala Asn Ser Ala Leu Asn Pro Ile Leu
305             310                 315                 320
Tyr Asn Met Thr Leu Cys Arg Asn Glu Trp Lys Lys Ile Phe Cys Cys
            325                 330                 335
Phe Trp Phe Pro Glu Lys Gly Ala Ile Leu Thr Asp Thr Ser Val Lys
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gcggtggaga caaccgtgct ggtgctcatc tttgcagtgt cgctgctggg caacgtgtgc 180
gccctggtgc tggtggcgcg ccgacgacgc cgcggcgcga ctgcctgcct ggtactcaac 240
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gtgcacctgc agcgcggcgt gcggggtcct gggcggcggg cgcgggcagt gctgctggcg 480
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ccgcaacggc tccccggcgc cgaccaggaa atttcgattt gcacactgat ttggcccacc 600
attcctggag agatctcgtg ggatgtctct tttgttactt tgaacttctt ggtgccagga 660
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ctcacggtaa gcctggccta ctcggagagc caccagatcc gcgtgtccca gcaggacttc 780
cggctcttcc gcaccctctt cctcctcatg gtctccttct tcatcatgtg gagcccatc 840
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tccctcttct tctgggtggt ggccttcaca tttgctaatt cagccctaaa ccccatcctc 960
tacaacatga cactgtgcag gaatgagtgg aagaaaattt tttgctgctt ctggttccca 1020
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tcgatgaccc tcttgacagc cacgagcgcg cgcagctccg ccatcttccc ggacgcgtgg 180
gccgggcgcc cggc atg tcc cct gag tgt gca cag acg acg ggc cct ggt  230
              Met Ser Pro Glu Cys Ala Gln Thr Thr Gly Pro Gly
               1           5                   10
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ccc tcg cac acc ctg gac caa gtc aat cgc acc cac ttc cct ttc ttc    278
Pro Ser His Thr Leu Asp Gln Val Asn Arg Thr His Phe Pro Phe Phe
        15              20                  25

tcg gat gtc aag ggc gac cac cgg ttg gtg ttg agc gtc gtg gag acc    326
Ser Asp Val Lys Gly Asp His Arg Leu Val Leu Ser Val Val Glu Thr
    30              35                  40

acc gtt ctg gga ctc atc ttt gtc gtc tca ctg ctg ggc aac gtg tgt    374
Thr Val Leu Gly Leu Ile Phe Val Val Ser Leu Leu Gly Asn Val Cys
45              50                  55                  60

gct cta gtg ctg gtg gcg cgc cgt cgg cgc cgt ggg gcg tca gcc agc    422
Ala Leu Val Leu Val Ala Arg Arg Arg Arg Arg Gly Ala Ser Ala Ser
                65                  70                  75

ctg gtg ctc aac ctc ttc tgc gcg gat ttg ctc ttc acc agc gcc atc    470
Leu Val Leu Asn Leu Phe Cys Ala Asp Leu Leu Phe Thr Ser Ala Ile
            80                  85                  90

cct cta gtg ctc gtc gtg cgc tgg act gag gcc tgg ctg ttg ggg ccc    518
Pro Leu Val Leu Val Val Arg Trp Thr Glu Ala Trp Leu Leu Gly Pro
            95                  100                 105

gtc gtc tgc cac ctg ctc ttc tac gtg atg aca atg agc ggc agc gtc    566
Val Val Cys His Leu Leu Phe Tyr Val Met Thr Met Ser Gly Ser Val
    110                 115                 120

acg atc ctc aca ctg gcc gcg gtc agc ctg gag cgc atg gtg tgc atc    614
Thr Ile Leu Thr Leu Ala Ala Val Ser Leu Glu Arg Met Val Cys Ile
125                 130                 135                 140

gtg cgc ctc cgg cgc ggc ttg agc ggc ccg ggg cgg cgg act cag gcg    662
Val Arg Leu Arg Arg Gly Leu Ser Gly Pro Gly Arg Arg Thr Gln Ala
                145                 150                 155

gca ctg ctg gct ttc ata tgg ggt tac tcg gcg ctc gcc gcg ctg ccc    710
Ala Leu Leu Ala Phe Ile Trp Gly Tyr Ser Ala Leu Ala Ala Leu Pro
            160                 165                 170

ctc tac atc ttg ttc cgc gtg gtc ccg cag cgc ctt ccc ggc ggg gac    758
Leu Tyr Ile Leu Phe Arg Val Val Pro Gln Arg Leu Pro Gly Gly Asp
            175                 180                 185

cag gaa att ccg att tgc aca ttg gat tgg ccc aac cgc ata gga gaa    806
Gln Glu Ile Pro Ile Cys Thr Leu Asp Trp Pro Asn Arg Ile Gly Glu
    190                 195                 200

atc tca tgg gat gtg ttt ttt gag act ttg aac ttc ctg gtg ccg gga    854
Ile Ser Trp Asp Val Phe Phe Glu Thr Leu Asn Phe Leu Val Pro Gly
205                 210                 215                 220

ctg gtc att gtg atc agt tac tcc aaa att tta cag atc acg aaa gca    902
Leu Val Ile Val Ile Ser Tyr Ser Lys Ile Leu Gln Ile Thr Lys Ala
                225                 230                 235
```

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tcg cgg aag agg ctt acg ctg agc ttg gca tac tct gag agc cac cag    950
Ser Arg Lys Arg Leu Thr Leu Ser Leu Ala Tyr Ser Glu Ser His Gln
            240             245             250

atc cga gtg tcc caa caa gac tac cga ctc ttc cgc acg ctc ttc ctg    998
Ile Arg Val Ser Gln Gln Asp Tyr Arg Leu Phe Arg Thr Leu Phe Leu
            255             260             265

ctc atg gtt tcc ttc ttc atc atg tgg agt ccc atc atc atc acc atc   1046
Leu Met Val Ser Phe Phe Ile Met Trp Ser Pro Ile Ile Ile Thr Ile
    270             275             280

ctc ctc atc ttg atc caa aac ttc cgg cag gac ctg gtc atc tgg cca   1094
Leu Leu Ile Leu Ile Gln Asn Phe Arg Gln Asp Leu Val Ile Trp Pro
285             290             295             300

tcc ctt ttc ttc tgg gtg gtg gcc ttc acg ttt gcc aac tct gcc cta   1142
Ser Leu Phe Phe Trp Val Val Ala Phe Thr Phe Ala Asn Ser Ala Leu
            305             310             315

aac ccc ata ctg tac aac atg tcg ctg ttc agg aac gaa tgg agg aag   1190
Asn Pro Ile Leu Tyr Asn Met Ser Leu Phe Arg Asn Glu Trp Arg Lys
            320             325             330

att ttt tgc tgc ttc ttt ttt cca gag aag gga gcc att ttt aca gat   1238
Ile Phe Cys Cys Phe Phe Phe Pro Glu Lys Gly Ala Ile Phe Thr Asp
            335             340             345

acg tct gtc agg cga aat gac ttg tct gtt att tcc agc taa           1280
Thr Ser Val Arg Arg Asn Asp Leu Ser Val Ile Ser Ser *
    350             355             360
```

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cagcaaatta aggaatgatc gctcagtata aaaatatttt tccttaaaag aactttctat 1460
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            20              25              30
Gly Asp His Arg Leu Val Leu Ser Val Val Glu Thr Thr Val Leu Gly
            35              40              45
Leu Ile Phe Val Val Ser Leu Leu Gly Asn Val Cys Ala Leu Val Leu
    50              55              60
Val Ala Arg Arg Arg Arg Arg Gly Ala Ser Ala Ser Leu Val Leu Asn
65              70              75              80
Leu Phe Cys Ala Asp Leu Leu Phe Thr Ser Ala Ile Pro Leu Val Leu
            85              90              95
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Val Val Arg Trp Thr Glu Ala Trp Leu Leu Gly Pro Val Val Cys His
        100                     105                 110
Leu Leu Phe Tyr Val Met Thr Met Ser Gly Ser Val Thr Ile Leu Thr
        115                     120                 125
Leu Ala Ala Val Ser Leu Glu Arg Met Val Cys Ile Val Arg Leu Arg
    130                     135                 140
Arg Gly Leu Ser Gly Pro Gly Arg Arg Thr Gln Ala Ala Leu Leu Ala
145                 150                     155                 160
Phe Ile Trp Gly Tyr Ser Ala Leu Ala Ala Leu Pro Leu Tyr Ile Leu
                165                     170                 175
Phe Arg Val Val Pro Gln Arg Leu Pro Gly Gly Asp Gln Glu Ile Pro
            180                     185                 190
Ile Cys Thr Leu Asp Trp Pro Asn Arg Ile Gly Glu Ile Ser Trp Asp
        195                     200                 205
Val Phe Phe Glu Thr Leu Asn Phe Leu Val Pro Gly Leu Val Ile Val
    210                     215                 220
Ile Ser Tyr Ser Lys Ile Leu Gln Ile Thr Lys Ala Ser Arg Lys Arg
225                     230                 235                 240
Leu Thr Leu Ser Leu Ala Tyr Ser Glu Ser His Gln Ile Arg Val Ser
                245                     250                 255
Gln Gln Asp Tyr Arg Leu Phe Arg Thr Leu Phe Leu Leu Met Val Ser
            260                     265                 270
Phe Phe Ile Met Trp Ser Pro Ile Ile Ile Thr Ile Leu Leu Ile Leu
        275                     280                 285
Ile Gln Asn Phe Arg Gln Asp Leu Val Ile Trp Pro Ser Leu Phe Phe
    290                     295                 300
Trp Val Val Ala Phe Thr Phe Ala Asn Ser Ala Leu Asn Pro Ile Leu
305                 310                     315                 320
Tyr Asn Met Ser Leu Phe Arg Asn Glu Trp Arg Lys Ile Phe Cys Cys
                325                     330                 335
Phe Phe Phe Pro Glu Lys Gly Ala Ile Phe Thr Asp Thr Ser Val Arg
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Arg Asn Asp Leu Ser Val Ile Ser Ser
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